(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification:
G01N 33/487 (2006.01)    A61B 5/00 (2006.01)
G01N 27/327 (2006.01)    G01N 27/30 (2006.01)

(21) International Application Number:
PCT/US2007/008491

(22) International Filing Date:    4 April 2007 (04.04.2007)

(25) Filing Language:    English

(26) Publication Language:    English

(30) Priority Data:
11/397,543    4 April 2006 (04.04.2006)    US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
—    without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS AND MATERIALS FOR CONTROLLING THE ELECTROCHEMISTRY OF ANALYTE SENSORS
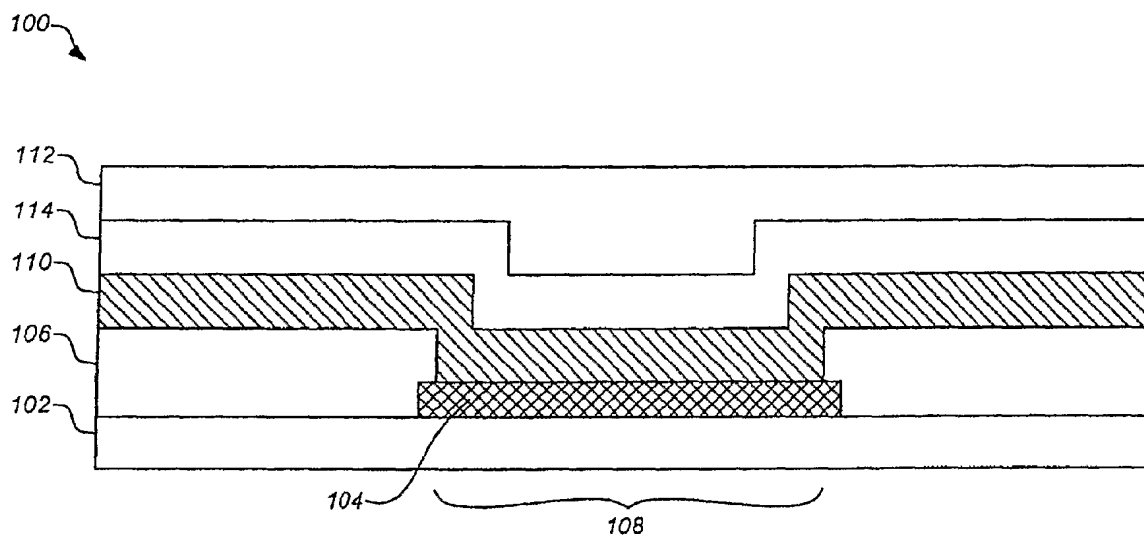
(57) Abstract: Embodiments of the invention provide electrochemical analyte sensors having elements designed to modulate their electrochemical reactions as well as methods for making and using such sensors.

# METHODS AND MATERIALS FOR CONTROLLING THE ELECTROCHEMISTRY OF ANALYTE SENSORS

## Cross Reference To Related Applications

5        This application is related to U.S. Patent Application Serial Number 10/273,767 filed October 18, 2002 (published as US-2004-0074785-A1), U.S. Patent Application Serial Number 10/861,837, filed June 4, 2004 and U.S. Patent Application Serial Number 11/301,512, filed December 13, 2005, the contents of each of which are incorporated herein by reference.

10                                        **Background of the Invention**

### 1. Field of the Invention.

        This invention relates to devices and methods for controlling the electrochemistry of analyte sensors such as glucose sensors used in the management of diabetes.

15      ### 2. Description of Related Art.

        Electrochemical measurements are widely used to determine the concentration of specific substances in fluids including biological analytes such as glucose and lactate. Maintaining the appropriate concentrations of glucose in the blood of an individual for example is extremely important for maintaining homeostasis. A concentration of glucose

20      below the normal range, or hypoglycemia, can cause unconsciousness and lowered blood pressure, and may even result in death. A concentration of glucose at levels higher than normal, or hyperglycemia, can result in synthesis of fatty acids and cholesterol, and in diabetics, coma. The measurement of the concentration of glucose in a person's blood, therefore, has become a necessity for diabetics who control the level of blood glucose by

25      insulin therapy.

        In clinical settings, accurate and relatively fast determinations of glucose and/or lactate levels can be determined from blood samples utilizing electrochemical sensors. In a typical electrochemical sensor, the analyte diffuses from the test environment into the sensor housing through a permeable membrane to a working electrode where the analyte

30      chemically reacts. A complementary chemical reaction occurs at a second electrode in the sensor housing known as a counter electrode. The electrochemical sensor produces

an analytical signal via the generation of a current arising directly from the oxidation or reduction of the analyte at the working and counter electrodes. In addition to a working electrode and a counter electrode, an electrolytic electrochemical sensor often includes a third electrode, commonly referred to as a reference electrode. A reference electrode is used to maintain the working electrode at a known voltage or potential.

In general, the electrodes of an electrochemical sensor provide a surface at which an oxidation or a reduction reaction occurs (that is, an electrochemically active surface) to provide a mechanism whereby the ionic conduction of an electrolyte solution in contact with the electrodes is coupled with the electron conduction of each electrode to provide a complete circuit for a current. By definition, the electrode at which an oxidation occurs is the anode, while the electrode at which the "complementary" reduction occurs is the cathode. In optimal sensors, the working and counter electrode in combination produce an electrical signal that is both related to the concentration of the analyte and is sufficiently strong to provide a signal-to-noise ratio suitable to distinguish between concentration levels of the analyte over the entire range of interest.

A common type of glucose or lactate electrode sensor comprises an enzyme electrode which utilizes an enzyme to convert glucose or lactate to an electroactive product which is then analyzed electrochemically. In such glucose sensors for example, a chemical reaction at the electrode converts glucose in the presence of enzymes, such as glucose oxidase, and results in the formation of reaction products including hydrogen peroxide. In these reactions, glucose reacts with oxygen to form gluconolactone and hydrogen peroxide. A suitable electrode can then measure the formation of hydrogen peroxide as an electrical signal. The signal is produced following the transfer of electrons from the peroxide to the electrode, and under suitable conditions, the enzyme catalyzed flow of current is proportional to the glucose concentration. Lactate electrode sensors including an enzyme electrode, similarly convert lactate in the presence of enzymes, such as lactate oxidase.

With respect to glucose sensors, in typical enzyme electrodes, glucose and oxygen from blood, as well as some interferants, such as ascorbic acid and uric acid, diffuse through a primary membrane of the sensor. As the glucose, oxygen and interferants

reach a second membrane, an enzyme, such as glucose oxidase, catalyzes the conversion of glucose to hydrogen peroxide and gluconolactone. The hydrogen peroxide may diffuse back through the primary membrane, or it may further diffuse through the secondary membrane to an electrode where it can be reacted to form oxygen and a

5    proton to produce a current proportional to the glucose concentration. While numerous devices for determination of glucose and lactate have been described, most of them have some limitations with respect to sensitivity, reproducibility, speed of response, number of effective uses, and/or the range of detection.

10                            **Summary of the Invention**

Embodiments of the invention disclosed herein include electrochemical analyte sensors comprising elements such as electrodes and/or electrode combinations (e.g. working and counter electrode combinations) designed to optimize factors including the reactivity, sensitivity, functioning and lifespan of the analyte sensors. An illustrative

15    embodiment of the invention is a method of performing an electrochemical reaction within an analyte sensor comprising using an analyte sensor constructed to include an electrode layer configuration that is designed to optimize the electrochemical reaction at the electrode when the electrode is exposed to an analyte. In such methods the analyte sensor typically includes at least one electrode disposed upon a base substrate where this

20    base substrate comprises a geometric feature selected to increase the surface area of an electrochemically reactive surface of the electrode disposed thereon such that surface area-to-volume ratio of the electrochemically-reactive surface area of the electrode disposed on the geometric feature is greater than surface-area-to-volume ratio of the reactive surface of the electrode when disposed on a flat surface. In some embodiments

25    of the invention, the surface area to volume ratio of the electrochemically reactive surface area of the electrode disposed on the geometric feature is at least 10%, 25%, 50%, 75% or 100% greater than surface area-to-volume ratio of the reactive surface of the electrode when disposed on a flat surface. In certain embodiments of the invention, an electrode in the analyte sensor comprises a porous matrix. In such embodiments, the

30    porous matrix can have a surface area that is at least 2, 4, 6, 8, 10, 12, 14, 16 or 18 times

the surface area of an essentially non-porous matrix of same dimensions.

Analyte sensors having electrodes constructed to have this type of configuration (e.g. where electrochemically reactive surface area of an electrode is selected to exhibit a geometry that has an electrochemically reactive surface area that is greater than if it were flat) can be constructed by a variety of methods known in the art; for example, by disposing the electrode material (e.g. a metal such as platinum) on a base substrate adjoining layer that includes a geometric feature comprising a lip, a shoulder, a ridge, a notch, a depression, a channel or the like. Typically, the geometric feature of the base substrate causes the electrochemically reactive surface area of the electrode to form a nodular geometry or the like.

In certain embodiments of the invention the analyte sensor comprises a plurality of discrete geometric features having a plurality of electrochemically reactive electrode surfaces. Such pluralities of features can include patterns such as rows of depressions and/or ridges or the like; for example, a row of ridges resembling zebra stripes. Optionally, the analyte sensor comprises at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 discrete geometric features having a plurality of electrochemically reactive electrode surfaces. In a specific embodiment of the invention, analyte sensor further comprises an analyte sensing layer disposed on the electrode having a relatively high surface area-to-volume ratio of the reactive surface, wherein the analyte sensing layer detectably alters the electrical current at the electrode in the presence of an analyte; an optional protein layer disposed on the analyte sensing layer; an adhesion promoting layer disposed on the analyte sensing layer or the optional protein layer, wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer; an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the diffusion of the analyte therethrough; and an optional cover layer disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer. In certain embodiments of the invention, the analyte sensor is designed to be implantable within a mammal. Optionally the electrochemical reaction at the electrode involves a protein reactive with an analyte

present in mammalian blood such as glucose oxidase, glucose dehydrogenase, lactate
oxidase, hexokinase or lactate dehydrogenase.

A related embodiment of the invention is an analyte sensor for detecting an
analyte in a fluid, the apparatus comprising at least one electrode disposed upon a base
5      substrate, wherein the base substrate includes a geometric feature selected to increase the
surface area of an electrochemically reactive surface on the electrode deposited thereon
(e.g. a lip, a shoulder, a ridge, a notch, a depression, a channel or the like) such that
surface-area-to-volume ratio of the electrochemically reactive surface area of the
electrode disposed on the geometric feature is greater than surface area-to-volume ratio
10     of the reactive surface of the electrode when disposed on a flat surface. Optionally, the
analyte sensor comprises a plurality of discrete geometric features having a plurality of
electrochemically reactive electrode surfaces. Typically, the analyte sensor is implantable
and comprises an analyte sensing layer disposed on the electrode, wherein the analyte
sensing layer detectably alters the electrical current at the electrode in the presence of an
15     analyte; an optional protein layer disposed on the analyte sensing layer; an adhesion
promoting layer disposed on the analyte sensing layer or the optional protein layer,
wherein the adhesion promoting layer promotes the adhesion between the analyte
sensing layer and an analyte modulating layer disposed on the analyte sensing layer; and
an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte
20     modulating layer modulates the diffusion of the analyte therethrough; and an optional
cover layer disposed on at least a portion of the analyte modulating layer, wherein the
cover layer further includes an aperture over at least a portion of the analyte modulating
layer. In certain embodiments of the invention, the implantable analyte sensor further
comprises an interference rejection layer disposed between the surface of the working
25     electrode and the analyte sensing layer.

Yet another embodiment of the invention is a method of modulating
electrochemical reactions within an implantable analyte sensor, the method comprising
performing electrochemical reactions within an implantable analyte sensor comprising a
working electrode having a reactive surface area, wherein during analyte sensing, the
30     working electrode generates electrons that reduce a plurality of composition species in

the electrochemical reaction including oxygen ($O_2$); and a counter electrode having a reactive surface area, wherein the size of the reactive surface area of the counter electrode is selected so as to control the reduction of the plurality of composition species in the electrochemical reaction so that oxygen ($O_2$) is the predominant composition

5    species reduced by the electrons generated at the working electrode, wherein the compound substrates having a first affinity for the electrons exhibit an affinity that is higher than the affinity of the compound substrates having a second affinity for the electrons; and a counter electrode of a second surface area, the second surface area is selected to be a size that reduces the interaction between electrons generated at the

10   working electrode with compound substrates having the second affinity for the electrons generated at the working electrode; so that electrochemical reactions within the implantable analyte sensor are modulated. In such methods the surface area of the counter electrode is typically about 1.5, 2, 2.5 or 3 times the size of the working electrode.

15       A related embodiment of the invention is an implantable electrochemical analyte sensor designed to include this electrode architecture. Optionally, the working electrode and the counter electrode in the analyte sensor comprise a porous matrix. Alternatively, the working electrode can comprise a relatively nonporous matrix while the counter electrode can comprise a porous matrix or vice versa. Optionally the porous matrix has a

20   surface area that is at least 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 10, 12, 14, 16 or 18 times the surface area of an essentially non-porous matrix of same dimensions. Optionally, the implantable analyte sensor further comprises an analyte sensing layer disposed on the working electrode, wherein the analyte sensing layer detectably alters the electrical current at the working electrode in the presence of an analyte; an optional

25   protein layer disposed on the analyte sensing layer; an adhesion promoting layer disposed on the analyte sensing layer or the optional protein layer, wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer; and an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the

30   diffusion of the analyte therethrough; and an optional cover layer disposed on at least a

portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer.

Yet another illustrative embodiment of the invention is a method of making a metallic electrode by electrodepositing a plurality of metal layers that comprise the electrode using cycles of differing electroplating conditions. Typically, the method comprises a first cycle of electroplating where a metal is electrodeposited onto a substrate under a first set of conditions selected to produce a first metal layer having a first surface area and a first adhesion strength between the substrate and the first metal layer. The method then involves a second cycle of electroplating where a metal composition is then electrodeposited onto the first metal layer under a second set of conditions selected to produce a second metal layer having a second surface area and a second adhesion strength between the first metal layer and the second metal layer. In this method, the first and second set of conditions are selected to produce a second metal layer having a second surface area that is greater than the first surface area of the first metal layer produced by the first set of conditions and a second metal layer having an adhesion with the first metal layer that is greater than the adhesion between the first metal layer and the substrate produced by the first set of conditions. Optionally, the method further comprises additional cycles of electroplating. In one such example, the method comprises a third cycle of electroplating where a metal composition is electrodeposited onto the second layer under a third set of conditions selected to produce a third metal layer having a third surface area. Typically, the second and third set of conditions are selected to produce a third metal layer having a greater density than the density of the second metal layer. In certain embodiments of the invention, the third set of conditions produces a third metal layer having a third surface area that is less than the second surface area of the second metal layer produced by the second set of conditions.

The invention also provides additional articles of manufacture including sensor elements, sensor sets and kits. In one such embodiment of the invention, a kit and/or sensor element or set, useful for the sensing an analyte as is described above, is provided. The kit and/or sensor set typically comprises a container, a label and a sensor as described above. The typical embodiment is a kit comprising a container and, within the

container, an analyte sensor apparatus having a design as disclosed herein and instructions for using the analyte sensor apparatus.

Other objects, features and advantages of the present invention will become apparent to those skilled in the art from the following detailed description. It is to be understood, however, that the detailed description and specific examples, while indicating some embodiments of the present invention are given by way of illustration and not limitation. Many changes and modifications within the scope of the present invention may be made without departing from the spirit thereof, and the invention includes all such modifications.

## Brief Description of the Figures

Figure 1 provides a schematic of the well known reaction between glucose and glucose oxidase. As shown in a stepwise manner, this reaction involves glucose oxidase (GOx), glucose and oxygen in water. In the reductive half of the reaction, two protons and electrons are transferred from β-D-glucose to the enzyme yielding d-gluconolactone. In the oxidative half of the reaction, the enzyme is oxidized by molecular oxygen yielding hydrogen peroxide. The d-gluconolactone then reacts with water to hydrolyze the lactone ring and produce gluconic acid. In certain electrochemical sensors of the invention, the hydrogen peroxide produced by this reaction is oxidized at the working electrode ($H_2O_2 \rightarrow 2H+ + O_2 + 2e^-$).

Figure 2 provides a diagrammatic view of a typical analyte sensor configuration of the current invention.

## Detailed Description of the Embodiments

Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the

art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols

5   and/or parameters unless otherwise noted. A number of terms are defined below.

The term "analyte" as used herein is a broad term and is used in its ordinary sense, including, without limitation, to refer to a substance or chemical constituent in a fluid such as a biological fluid (for example, blood, interstitial fluid, cerebral spinal fluid, lymph fluid or urine) that can be analyzed. Analytes can include naturally occurring

10  substances, artificial substances, metabolites, and/or reaction products. In some embodiments, the analyte for measurement by the sensing regions, devices, and methods is glucose. However, other analytes are contemplated as well, including but not limited to, lactate. Salts, sugars, proteins fats, vitamins and hormones naturally occurring in blood or interstitial fluids can constitute analytes in certain embodiments. The analyte

15  can be naturally present in the biological fluid or endogenous; for example, a metabolic product, a hormone, an antigen, an antibody, and the like. Alternatively, the analyte can be introduced into the body or exogenous, for example, a contrast agent for imaging, a radioisotope, a chemical agent, a fluorocarbon-based synthetic blood, or a drug or pharmaceutical composition, including but not limited to insulin. The metabolic

20  products of drugs and pharmaceutical compositions are also contemplated analytes.

The term "sensor," as used herein, is a broad term and is used in its ordinary sense, including, without limitation, the portion or portions of an analyte-monitoring device that detects an analyte. In one embodiment, the sensor includes an electrochemical cell that has a working electrode, a reference electrode, and optionally a

25  counter electrode passing through and secured within the sensor body forming an electrochemically reactive surface at one location on the body, an electronic connection at another location on the body, and a membrane system affixed to the body and covering the electrochemically reactive surface. During general operation of the sensor, a biological sample (for example, blood or interstitial fluid), or a portion thereof, contacts

30  (directly or after passage through one or more membranes or domains) an enzyme (for

example, glucose oxidase); the reaction of the biological sample (or portion thereof) results in the formation of reaction products that allow a determination of the analyte level in the biological sample.

The term "electrochemical cell," as used herein, is a broad term and is used in its

5   ordinary sense, including, without limitation, a device in which chemical energy is converted to electrical energy. Such a cell typically consists of two or more electrodes held apart from each other and in contact with an electrolyte solution. Connection of the electrodes to a source of direct electric current renders one of them negatively charged and the other positively charged. Positive ions in the electrolyte migrate to the

10  negative electrode (cathode) and there combine with one or more electrons, losing part or all of their charge and becoming new ions having lower charge or neutral atoms or molecules; at the same time, negative ions migrate to the positive electrode (anode) and transfer one or more electrons to it, also becoming new ions or neutral particles. The overall effect of the two processes is the transfer of electrons from the negative ions to

15  the positive ions, a chemical reaction.

The terms "electrochemically reactive surface" and "electroactive surface" as used herein are broad terms and are used in their ordinary sense, including, without limitation, the surface of an electrode where an electrochemical reaction takes place. In one example, a working electrode measures hydrogen peroxide produced by the enzyme

20  catalyzed reaction of the analyte being detected reacts creating an electric current (for example, detection of glucose analyte utilizing glucose oxidase produces $H_2O_2$ as a by product, $H_2O_2$ reacts with the surface of the working electrode producing two protons ($2H^+$), two electrons ($2e^-$) and one molecule of oxygen ($O_2$) which produces the electronic current being detected). In the case of the counter electrode, a reducible

25  species, for example, $O_2$ is reduced at the electrode surface in order to balance the current being generated by the working electrode.

The term "sensing region" as used herein is a broad term and is used in its ordinary sense, including, without limitation, the region of a monitoring device responsible for the detection of a particular analyte. In an illustrative embodiment, the

30  sensing region can comprise a non-conductive body, a working electrode, a reference

electrode, and a counter electrode passing through and secured within the body forming electrochemically reactive surfaces on the body and an electronic connective means at another location on the body, and a one or more layers covering the electrochemically reactive surface.

5      The terms "electrical potential" and "potential" as used herein, are broad terms and are used in their ordinary sense, including, without limitation, the electrical potential difference between two points in a circuit which is the cause of the flow of a current. The term "system noise," as used herein, is a broad term and is used in its ordinary sense, including, without limitation, unwanted electronic or diffusion-related noise which can

10     include Gaussian, motion-related, flicker, kinetic, or other white noise, for example.

The terms "interferants" and "interfering species," as used herein, are broad terms and are used in their ordinary sense, including, but not limited to, effects and/or species that interfere with the measurement of an analyte of interest in a sensor to produce a signal that does not accurately represent the analyte measurement. In one

15     example of an electrochemical sensor, interfering species are compounds with an oxidation potential that overlaps with the analyte to be measured.

As discussed in detail below, embodiments of the invention relate to the use of an electrochemical sensor that measures a concentration of an analyte of interest or a substance indicative of the concentration or presence of the analyte in fluid. In some

20     embodiments, the sensor is a continuous device, for example a subcutaneous, transdermal, or intravascular device. In some embodiments, the device can analyze a plurality of intermittent blood samples. The sensor embodiments disclosed herein can use any known method, including invasive, minimally invasive, and non-invasive sensing techniques, to provide an output signal indicative of the concentration of the analyte of

25     interest. Typically, the sensor is of the type that senses a product or reactant of an enzymatic reaction between an analyte and an enzyme in the presence of oxygen as a measure of the analyte in vivo or in vitro. Such sensors typically comprise a membrane surrounding the enzyme through which a bodily fluid passes and in which an analyte within the bodily fluid reacts with an enzyme in the presence of oxygen to generate a

30     product. The product is then measured using electrochemical methods and thus the

output of an electrode system functions as a measure of the analyte. In some
embodiments, the sensor can use an amperometric, coulometric, conductimetric, and/or
potentiometric technique for measuring the analyte.

Embodiments of the invention described herein can be adapted and
5    implemented with a wide variety of known electrochemical sensors, including for
example, U.S. Patent Application No. 20050115832, U.S. Pat. Nos. 6,001,067, 6,702,857,
6,212,416, 6,119,028, 6,400,974, 6,595,919, 6,141,573, 6,122,536, 6,512,939 5,605,152,
4,431,004, 4,703,756, 6,514,718, 5,985,129, 5,390,691, 5,391, 250, 5,482,473, 5,299,571,
5,568,806, 5,494,562, 6,120,676, 6,542,765 as well as PCT International Publication
10   Numbers WO 01/58348, WO 04/021877, WO 03/034902, WO 03/035117, WO
03/035891, WO 03/023388, WO 03/022128, WO 03/022352, WO 03/023708, WO
03/036255, WO03/036310 and WO 03/074107, and European Patent Application EP
1153571, the contents of each of which are incorporated herein by reference.

Embodiments of the invention disclosed herein provide sensors of the type used,
15   for example, in subcutaneous or transcutaneous monitoring of blood glucose levels in a
diabetic patient. A variety of implantable, electrochemical biosensors have been
developed for the treatment of diabetes and other life-threatening diseases. Many
existing sensor designs use some form of immobilized enzyme to achieve their bio-
specificity. For example, a first class of glucose sensor designs use a very thin (<1
20   micron) layer of glucose oxidase (GOx) and bovine serum albumin that is either spray or
spin coated onto the working electrode and cross-linked with glutaraldehyde.
Alternatively, a second class of glucose sensor design employs a thick (~1 mm) hydrogel
known as the Sensor Matrix Protein (SMP), which typically consists of an enzyme such
as GOx and human serum albumin cross-linked together with a cross-linking agent such
25   as glutaraldehyde. Relative to each other, the immobilized enzyme configurations of the
two above-noted classes of sensor designs possess different advantages that serve to
increase operational sensor life. Due to the close proximity of the immobilized GOx to
the peroxide-consuming electrode, the first class of sensor designs are believed to
possess significantly decreased enzyme deactivation rate constants. In comparison, the
30   thick SMPs utilized in the second class of sensor designs can incorporate orders of

magnitude more enzyme than the first class.

Many sensor designs utilize a matrix (or a plurality of matrices) such as an enzymatic hydrogel matrix to function. The term "matrix" is used herein according to its art-accepted meaning of something within or from which something else originates,

5      develops, takes form and/or is found. An exemplary enzymatic hydrogel matrix for example typically comprises a bio-sensing enzyme (e.g. glucose oxidase or lactate oxidase) and human serum albumin proteins that have been cross-linked together with a crosslinking agent such as glutaraldehyde to form a polymer network. This network is then swollen with an aqueous solution to form an enzymatic hydrogel matrix. The

10     degree of swelling of this hydrogel frequently increases over a time-period of several weeks, and is presumably due to the degradation of network cross-links. Regardless of its cause, an observed consequence of this swelling is the protrusion of the hydrogel outside of the hole or "window" cut into the outer sensor tubing. This causes the sensor dimensions to exceed design specifications and has a negative impact on its analytical

15     performance.

Embodiments of the invention disclosed herein provide sensor elements having enhanced material properties and sensors constructed from such elements. The disclosure further provides methods for making and using such sensors. While some embodiments of the invention pertain to glucose and/or lactate sensors, a variety of the

20     elements disclosed herein (e.g. electrodes and electrode designs) can be adapted for use with any one of the wide variety of sensors known in the art. The analyte sensor elements, architectures and methods for making and using these elements that are disclosed herein can be used to establish a variety of layered sensor structures. Such sensors of the invention exhibit a surprising degree of flexibility and versatility,

25     characteristic which allow a wide variety of sensor configurations to be designed to examine a wide variety of analyte species.

In typical embodiments of the present invention, the transduction of the analyte concentration into a processable signal is by electrochemical means. These transducers may include any of a wide variety of amperometric, potentiometric, or conductimetric

30     base sensors known in the art. Moreover, the microfabrication sensor techniques and

materials of the instant invention may be applied to other types of transducers (e.g., acoustic wave sensing devices, thermistors, gas-sensing electrodes, field-effect transistors, optical and evanescent field wave guides, and the like) fabricated in a substantially nonplanar, or alternatively, a substantially planar manner. A useful discussion and
5    tabulation of transducers which may be exploited in a biosensor as well as the kinds of analytical applications in which each type of transducer or biosensor, in general, may be utilized, is found in an article by Christopher R. Lowe in Trends in Biotech. 1984, 2(3), 59-65.

Specific aspects of the invention are discussed in detail in the following sections.
10

I.      TYPICAL ELEMENTS, CONFIGURATIONS AND ANALYTE
        SENSORS OF THE INVENTION

A.      OPTIMIZED SENSOR ELEMENTS OF THE INVENTION
15           Embodiments of the sensors disclosed herein incorporate one or more sensor elements having enhanced material properties. Embodiments of the invention include sensor including these elements and well methods for making and using them. Embodiments of the invention disclosed herein include electrochemical analyte sensors comprising elements such as electrodes and/or electrode combinations (e.g. working an
20   counter electrode combinations) designed to optimize factors including the reactivity, sensitivity, functioning and lifespan of the analyte sensors. Certain specific embodiments of the invention are designed to optimize the electrochemical reactions that function in the sensing of an analyte of interest. The optimized embodiments of the invention disclosed herein can be utilized and/or applied to a wide variety of sensor methods and
25   designs. The following sections describe illustrative sensor elements, sensor configurations and methodological embodiments of the invention.

Certain embodiments of the invention are designed to enhance sensor stability. As discussed in detail below, embodiments of the invention include analyte sensors having a plurality of adjoining layers consisting of different functional constituents. As is
30   known in the art, with certain sensors that include a plurality of layers, one or more of the layers can become unstable and separate in part or in whole from an adjoining layer.

Such delamination events can compromise the function of one or more of the different constituents and consequently have a negative impact on a sensor's function, for example its long term stability. In this context, we observe that, with certain sensor embodiments a series of flat, uniform layers typically have better adherence between layers and are

5    therefore more stable than sensors having disparate layers of rough and/or polymorphic constituents.

One example of layer that can exhibit problems with delamination is an electrode layer that is deposited upon a base layer in a manner that creates an irregular surface, for example when a platinum electrode layer is deposited on a base substrate that has an

10   edge or shoulder that causes a nodule of a electrode material (e.g. platinum black) to form over this feature on the base substrate. This can happen, for example, when platinum black is disposed in the sensor in a manner that allows it to grow up the side of a material already in the sensor; for example, an edge of an insulating composition. In this context, one embodiment of the invention is a method of contributing to the

15   uniformity of a layer in an analyte sensing device comprising a plurality of layers, the method comprising disposing an electrode used in the device on a base substrate, wherein the base substrate is selected to exhibit an essentially or predominantly flat geometry so that the electrode disposed on the base substrate also exhibits a relatively flat geometry, thereby contributing to the uniformity of a layer in a analyte sensing

20   device. In this method, the sensor can be manufactured according to a process that avoids depositing the electrode materials on geometric features already present in the sensor. Optionally, the base substrate is selected to exhibit a flat geometry by avoiding disposing the electrode on a ridge, lip, shoulder or edge generated by an insulating composition that is disposed in an area where the electrode is disposed.

25   A related embodiment of the invention is a method of contributing to the uniformity of a layer in an analyte sensing device comprising a plurality of layers, the method including disposing an electrode used in the device on a base substrate, and then removing a portion of the electrode so disposed on the base substrate, wherein the portion of the electrode disposed on the base substrate that is removed is an irregular

30   feature such as a bulge or the like that results from the electrode being disposed on a lip,

shoulder, edge or the like etc. that is present in another of the plurality of layers that is
disposed in an area where the electrode is disposed. In this method, the resulting layer
having the electrode where the bulge or nodule is removed exhibits a more uniform
surface than does the layer where the bulge is not removed. Such bulges can be removed

5      by one of a variety of methods known in the art, for example, via mechanical or chemical
processes. Typically, these methods of contributing to the uniformity of one or more
layers in an analyte sensor have the result of inhibiting delamination of a layer within the
plurality of layers. These methods of contributing to the uniformity of one or more
layers in an analyte sensor can also increase sensor stability; for example, the in vivo

10     stability of a sensor.

In view of the reasons noted above, those of skill in this art familiar with this
phenomena would be motivated to construct sensors having a plurality of uniform layers.
However, as disclosed herein, it has been discovered that in certain contexts, sensors
having electrode surfaces with unusual and/or non-uniform geometries have unexpected

15     and desirable properties. In particular, sensors designed so that the electrode material
(e.g. platinum) is deposited on a base substrate that includes a geometric feature selected
to increase the surface area of an electrochemically reactive surface on the electrode
exhibit a number of beneficial properties, such as the ability to generate a greater signal
in response to analyte, a reduced signal to noise ratio and a greater longevity, in

20     particular, a greater longevity in vivo. Surprisingly, in certain embodiments of the
invention, the beneficial properties of these shaped electrodes outweigh the detrimental
effects that result from a lack of uniformity in the electrode layer. This unexpected
observation forms the basis of certain embodiments of the invention discussed below.

One illustrative embodiment of the invention is a method of performing an

25     electrochemical reaction within an analyte sensor comprising using an analyte sensor
constructed to include an electrode layer configuration that is designed to optimize the
electrochemical reaction at the electrode when the electrode is exposed to an analyte. In
such methods the analyte sensor typically includes at least one electrode disposed upon a
base substrate where this base substrate comprises a geometric feature selected to

30     increase the surface area of an electrochemically reactive surface of the electrode

disposed thereon such that the surface area-to-volume ratio of the electrochemically
reactive surface area of the electrode disposed on the geometric feature is greater than
surface area-to-volume ratio of the reactive surface of the electrode when disposed on a
flat surface. Optionally, the electrode further comprises a porous matrix. In certain

5      embodiments, the porous matrix has a surface area that is at least 2, 4, 6, 8, 10, 12, 14, 16
or 18 times the surface area of an essentially non-porous matrix of same dimensions.

In certain methodological embodiments of the invention, exposing the analyte
sensor to an analyte so that an electrochemical reaction is performed within an analyte
sensor having electrodes constructed to have this type of configuration, the electronic

10     signal in response to exposure to the analyte that is generated at the electrochemically
reactive surface area of the electrode disposed on the geometric feature is greater than
the electronic signal generated an electrochemically reactive surface area of the electrode
when the electrode is disposed on a flat surface. Similarly, in certain methodological
embodiments of the invention, exposing the analyte sensor to an analyte so that an

15     electrochemical reaction is performed within an analyte sensor having electrodes
constructed to have this type of configuration, the in vivo lifetime of the analyte sensor
having the electrochemically reactive surface area of the electrode disposed on the
geometric feature is greater than the in vivo lifetime of an analyte sensor having an
electronic signal generated on an electrochemically reactive surface area of an electrode

20     when the electrode is disposed on a flat surface.

While not being bound by a specific scientific theory, it is believed that the highly
desirable properties that are observed with analyte sensors constructed to have more
complex geometries are due to a relative increase in the size of the electrochemically
reactive surface area of these electrodes. In particular, embodiments of the invention

25     using such electrode geometries are believed to generate a larger electrochemically
reactive surface area for reacting with and sensing analytes while the amount of total
space occupied by the electrode layer within the sensor remains essentially unchanged or
changes very little. This allows for a large surface area in a small space. A similar
phenomena is observed in human brains, organs that are highly folded to generate a large

30     surface area within a small space. In particular, the cerebral cortex of the human brain is

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highly convoluted, meaning it has many folds and creases, convolutions that allow a large surface area of brain (and an associated larger number of neurons) to fit inside our relatively small skulls. In some embodiments of the invention, the surface area to volume ratio of the electrochemically reactive surface area of the electrode disposed on

5     the geometric feature is at least 10%, 25%, 50%, 75% or 100% greater than surface area-to-volume ratio of the reactive surface of the electrode when disposed on a flat surface.

Analyte sensor having electrodes constructed to have this type of configuration (e.g. where electrochemically reactive surface area of an electrode is disposed on the geometric feature so that the electrochemically reactive surface area is greater than if it

10   was disposed on a flat surface) can be constructed by a variety of methods known in the art, for example by disposing the electrode material (e.g. a metal such as platinum) on a base substrate adjoining layer that includes a geometric feature comprising a lip, a shoulder, a ridge, a notch, a depression, a channel or the like. Typically, the geometric feature of the base substrate causes the electrochemically reactive surface area of the

15   electrode to form a nodule or the like. While the deposition of an electrode onto a base substrate is one way to generate an electrode with a high surface area to volume ratio, electrodes having these properties can be generated by other processes known in the art. For example, in an alternative embodiment of the invention, electrodes having a high surface area to volume ratio can be premade and then subsequently disposed within the

20   analyte sensor.

In certain embodiments of the invention the analyte sensor comprises a plurality of discrete geometric features having a plurality of electrochemically reactive electrode surfaces. Such pluralities of features can include patterns such as rows of depressions and/or ridges or the like, for example a row of ridges resembling zebra stripes. In some

25   embodiments of the invention, the sensor is manufactured to include these pluralities of features on which the electrode material can be deposited. Optionally, the analyte sensor comprises at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 discrete geometric features having a plurality of electrochemically reactive electrode surfaces. In a specific embodiment of the invention, analyte sensor further comprises an analyte sensing layer disposed on the

30   electrode having a relatively high surface area-to-volume ratio of the reactive surface,

wherein the analyte sensing layer detectably alters the electrical current at the electrode in the presence of an analyte; an optional protein layer disposed on the analyte sensing layer; an adhesion promoting layer disposed on the analyte sensing layer or the optional protein layer, wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer; and an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the diffusion of the analyte therethrough; and an optional cover layer disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer. In certain embodiments of the invention, the analyte sensor is designed to be implantable within a mammal. Optionally the electrochemical reaction at the electrode involves a protein reactive with an analyte present in mammalian blood such as glucose oxidase, glucose dehydrogenase, lactate oxidase, hexokinase or lactate dehydrogenase. In some embodiments of the invention, in the electrochemical reaction, hydrogen peroxide is oxidized at the electrochemically reactive surface area of the electrode disposed on the geometric feature of the base substrate.

A related embodiment of the invention is an analyte sensor for detecting an analyte in a fluid, the apparatus comprising at least one electrode disposed upon a base substrate, wherein the base substrate includes a geometric feature selected to increase the surface area of an electrochemically reactive surface on the electrode deposited thereon (e.g. a lip, a shoulder, a ridge, a notch, a depression, a channel or the like) such that surface area-to-volume ratio of the electrochemically reactive surface area of the electrode disposed on the geometric feature is greater than surface area-to-volume ratio of the reactive surface of the electrode when disposed on a flat surface. Optionally, the analyte sensor comprises a plurality of discrete geometric features having a plurality of electrochemically reactive electrode surfaces. Typically, the analyte sensor is implantable and comprises an analyte sensing layer disposed on the electrode, wherein the analyte sensing layer detectably alters the electrical current at the electrode in the presence of an analyte; an optional protein layer disposed on the analyte sensing layer; an adhesion promoting layer disposed on the analyte sensing layer or the optional protein layer,

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wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer; and an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the diffusion of the analyte therethrough; and an optional

5      cover layer disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer. In certain embodiments of the invention, the implantable analyte sensor further comprises an interference rejection layer disposed between the surface of the working electrode and the analyte sensing layer.

10     Yet another embodiment of the invention is a method of modulating electrochemical reactions within an implantable analyte sensor, the method comprising performing electrochemical reactions within an implantable analyte sensor comprising: a working electrode having a first surface area, wherein electrochemical reactions at the working electrode generate electrons that interact with compound substrates having a

15     first affinity for the electrons that is greater than or equal to that of oxygen ($O_2$), and compound substrates having a second affinity for the electrons that is less than or equal to that of oxygen ($O_2$), wherein the second surface area is selected to be a size that reduces the interaction between electrons generated at the working electrode with compound substrates having the second affinity for the electrons generated at the

20     working electrode; so that electrochemical reactions within the implantable analyte sensor are modulated.

A closely related embodiment of the invention is a method of modulating electrochemical reactions within an implantable analyte sensor, the method comprising performing electrochemical reactions within an implantable analyte sensor comprising: a

25     working electrode having a reactive surface area, wherein during analyte sensing, the working electrode generates electrons that reduce a plurality of composition species in the electrochemical reaction including oxygen ($O_2$); and a counter electrode having a reactive surface area, wherein the size of the reactive surface area of the counter electrode (e.g. relative to the size of the reactive surface area of the working electrode) is

30     selected so as to control the reduction of the plurality of composition species in the

electrochemical reaction so that oxygen ($O_2$) is the predominant composition species reduced by the electrons generated at the working electrode over the functional lifetime of the sensor, so that electrochemical reactions within the implantable analyte sensor are modulated over the lifetime of the sensor. The term predominant is used herein

5     according to its art accepted meaning of being the most frequent or common. In one illustrative example of such an embodiment, 51% of the electrons generated at the working electrode reduce oxygen ($O_2$) species over the lifetime of the sensor.

     The methods (and associated sensor architectures) designed to selectively reduce oxygen ($O_2$) species in the electrochemical reactions have a number of surprising

10    advantages over existing embodiments in the art. By constructing sensors with elements specifically selected so that the size of the reactive surface area of the counter electrode relative to the size of the reactive surface area of the working electrode controls the reduction of the plurality of composition species, the sensing of the analyte is therefore occurs in a controlled environment. In this way, this sensor structure maintains a greater

15    consistency in the electrochemical reaction environment (leading to a greater consistency in signals generated by the electrochemical reactions) during the period that the analyte sensor is in use. For example, when the analyte sensors employ a oxidase in analyte sensing such as the protein glucose oxidase, using this sensor structure to control the electrochemical reaction so that oxygen ($O_2$) is the predominant composition species

20    reduced by the electrons generated at the working electrode over the functional lifetime of the sensor provides a benefit by providing a stabilized reactive environment for the electrochemical reactions that function to provide the sensor signal.

     While analyte sensors having working and counter electrodes of different sizes may be described in the art, the art fails to teach or suggest the invention described

25    herein, e.g. sensors constructed to include elements specifically designed to control the electrochemical reactions in the described manner (i.e. so that oxygen ($O_2$) is the predominant composition species reduced by electrons generated at the working electrode). In addition, embodiments of the sensors disclosed herein are designed to vary the size of the reactive surface area of the counter electrode relative to the size of

30    the reactive surface area of the working electrode, and not just the size of the electrodes.

For example, in certain embodiments of the invention, the counter and working electrodes can be of approximately the same size. In such embodiments, the counter electrode can be constructed to exhibit an architecture that provides a greater surface area/size ratio (e.g. one constructed using a porous matrix) so that the greater size of the

5      reactive surface area of the counter electrode (e.g. relative to the size of the reactive surface area of the working electrode) controls the reduction of the plurality of composition species in the electrochemical reaction so that oxygen ($O_2$) is the predominant composition species reduced by the electrons generated at the working electrode.

10      Optionally, the working electrode and the counter electrode in analyte sensors having the above-noted architectures comprise a porous matrix. Alternatively, the working electrode comprises a relatively nonporous matrix while the counter electrode comprises a porous matrix or vice versa. Optionally the porous matrix has a surface area that is at least 2, 4, 6, 8, 10, 12, 14, 16 or 18 times the surface area of an essentially non-

15      porous matrix of same dimensions. In such methods the surface area of the counter electrode is typically about 1.5, 2, 2.5 or 3 times the size of the working electrode. Optionally, the implantable analyte sensor comprises an analyte sensing layer disposed on the working electrode, wherein the analyte sensing layer detectably alters the electrical current at the working electrode in the presence of an analyte; an optional protein layer

20      disposed on the analyte sensing layer; an adhesion promoting layer disposed on the analyte sensing layer or the optional protein layer, wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer; and an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the diffusion of the

25      analyte therethrough; and an optional cover layer disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer.

A related embodiment of the invention is an implantable electrochemical analyte sensor comprising a working electrode having a first surface area, wherein

30      electrochemical reactions at the working electrode generate electrons that interact with

compound substrates having a first affinity for the electrons and compound substrates having a second affinity for the electrons, wherein the compound substrates having a first affinity for the electrons exhibit an affinity that is higher than the affinity of the compound substrates having a second affinity for the electrons; and a counter electrode

5   of a second surface area, the second surface area is selected to be a size that reduces the interaction between electrons generated at the working electrode with compound substrates having the second affinity for the electrons generated at the working electrode; an analyte sensing layer disposed on the working electrode, wherein the analyte sensing layer detectably alters the electrical current at the working electrode in the presence of an

10  analyte; an adhesion promoting layer disposed on the analyte sensing layer or the protein layer, wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer; an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the diffusion of the analyte therethrough; and a cover layer

15  disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer.

**B.    DIAGRAMMATIC ILLUSTRATION OF TYPICAL SENSOR CONFIGURATIONS**

20
    FIG. 2 illustrates a cross-section of a typical sensor structure 100 of the present invention. The sensor is formed from a plurality of components that are typically in the form of layers of various conductive and non-conductive constituents disposed on each other according to a method of the invention to produce a sensor structure. The

25  components of the sensor are typically characterized herein as layers because, for example, it allows for a facile characterization of the sensor structure shown in FIG. 2. Artisans will understand however, that in certain embodiments of the invention, the sensor constituents are combined such that multiple constituents form one or more heterogeneous layers. In this context, those of skill in the art understand that the

30  ordering of the layered constituents can be altered in various embodiments of the invention.

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The embodiment shown in FIG. 2 includes a base layer 102 to support the sensor 100. The base layer 102 can be made of a material such as a metal and/or a ceramic and/or a polymeric substrate, which may be self-supporting or further supported by another material as is known in the art. Embodiments of the invention include a

5    conductive layer 104 which is disposed on and/or combined with the base layer 102. In certain embodiments, the base layer 102 and/or the conductive layer 104 can be constructed to produce electrodes having a configuration where the electrochemically reactive surface area of an electrode is disposed on the geometric feature so that the electrochemically reactive surface area is greater than if it was disposed on a flat surface.

10    Typically the conductive layer 104 comprises one or more electrodes. An operating sensor 100 typically includes a plurality of electrodes such as a working electrode, a counter electrode and a reference electrode. Other embodiments may also include an electrode that performs multiple functions, for example one that functions as both as a reference and a counter electrode. Still other embodiments may utilize a

15    separate reference element not formed on the sensor. Typically these electrodes are electrically isolated from each other, while situated in close proximity to one another.

As discussed in detail below, the base layer 102 and/or conductive layer 104 can be generated using many known techniques and materials. In certain embodiments of the invention, the electrical circuit of the sensor is defined by etching the disposed

20    conductive layer 104 into a desired pattern of conductive paths. A typical electrical circuit for the sensor 100 comprises two or more adjacent conductive paths with regions at a proximal end to form contact pads and regions at a distal end to form sensor electrodes. An electrically insulating cover layer 106 such as a polymer coating is optionally disposed on portions of the sensor 100. Acceptable polymer coatings for use

25    as the insulating protective cover layer 106 can include, but are not limited to, non-toxic biocompatible polymers such as silicone compounds, polyimides, biocompatible solder masks, epoxy acrylate copolymers, or the like. In the sensors of the present invention, one or more exposed regions or apertures 108 can be made through the cover layer 106 to open the conductive layer 104 to the external environment and to, for example, allow

30    an analyte such as glucose to permeate the layers of the sensor and be sensed by the

sensing elements. Apertures 108 can be formed by a number of techniques, including laser ablation, tape masking, chemical milling or etching or photolithographic development or the like. In certain embodiments of the invention, during manufacture, a secondary photoresist can also be applied to the protective layer 106 to define the

5   regions of the protective layer to be removed to form the aperture(s) 108. The exposed electrodes and/or contact pads can also undergo secondary processing (e.g. through the apertures 108), such as additional plating processing, to prepare the surfaces and/or strengthen the conductive regions.

In the sensor configuration shown in FIG. 2, an analyte sensing layer 110 (which

10  is typically a sensor chemistry layer, meaning that materials in this layer undergo a chemical reaction to produce a signal that can be sensed by the conductive layer) is disposed on one or more of the exposed electrodes of the conductive layer 104. Typically, the sensor chemistry layer 110 is an enzyme layer. Most typically, the sensor chemistry layer 110 comprises an enzyme capable of producing and/or utilizing oxygen

15  and/or hydrogen peroxide, for example the enzyme glucose oxidase. Optionally the enzyme in the sensor chemistry layer is combined with a second carrier protein such as human serum albumin, bovine serum albumin or the like. In an illustrative embodiment, an enzyme such as glucose oxidase in the sensor chemistry layer 110 reacts with glucose to produce hydrogen peroxide, a compound which then modulates a current at an

20  electrode. As this modulation of current depends on the concentration of hydrogen peroxide, and the concentration of hydrogen peroxide correlates to the concentration of glucose, the concentration of glucose can be determined by monitoring this modulation in the current. In a specific embodiment of the invention, the hydrogen peroxide is oxidized at a working electrode which is an anode (also termed herein the anodic

25  working electrode), with the resulting current being proportional to the hydrogen peroxide concentration. Such modulations in the current caused by changing hydrogen peroxide concentrations can by monitored by any one of a variety of sensor detector apparatuses such as a universal sensor amperometric biosensor detector or one of the other variety of similar devices known in the art such as glucose monitoring devices

30  produced by Medtronic MiniMed.

The analyte sensing layer 110 can be applied over portions of the conductive
layer or over the entire region of the conductive layer. Typically the analyte sensing layer
110 is disposed on the working electrode which can be the anode or the cathode.
Optionally, the analyte sensing layer 110 is also disposed on a counter and/or reference

5    electrode. While the analyte sensing layer 110 can be up to about 1000 microns ($\mu$m) in
thickness, typically the analyte sensing layer is relatively thin as compared to those found
in sensors previously described in the art, and is for example, typically less than 1, 0.5,
0.25 or 0.1 microns in thickness. As discussed in detail below, some methods for
generating a thin analyte sensing layer 110 include spin coating processes, dip and dry

10   processes, low shear spraying processes, ink-jet printing processes, silk screen processes
and the like. Most typically the thin analyte sensing layer 110 is applied using a spin
coating process.

Typically, the analyte sensing layer 110 is coated with one or more additional
layers. Optionally, the one or more additional layers includes a protein layer 116

15   disposed upon the analyte sensing layer 110. Typically, the protein layer 116 comprises a
protein such as albumin or the like. Typically, the protein layer 116 comprises human
serum albumin. In some embodiments of the invention, an additional layer includes an
analyte modulating layer 112 that is disposed above the analyte sensing layer 110 to
regulate analyte contact with the analyte sensing layer 110. For example, the analyte

20   modulating membrane layer 112 can comprise a glucose limiting membrane, which
regulates the amount of glucose that contacts an enzyme such as glucose oxidase that is
present in the analyte sensing layer. Such glucose limiting membranes can be made from
a wide variety of materials known to be suitable for such purposes, e.g., silicone
compounds such as polydimethyl siloxanes, polyurethanes, polyurea cellulose acetates,

25   Nafion, polyester sulfonic acids (e.g. Kodak AQ), hydrogels or any other suitable
hydrophilic membranes known to those skilled in the art.

In typical embodiments of the invention, an adhesion promoter layer 114 is
disposed between the analyte modulating layer 112 and the analyte sensing layer 110 as
shown in FIG. 2 in order to facilitate their contact and/or adhesion. In a specific

30   embodiment of the invention, an adhesion promoter layer 114 is disposed between the

analyte modulating layer 112 and the protein layer 116 as shown in FIG. 2 in order to

facilitate their contact and/or adhesion. The adhesion promoter layer 114 can be made

from any one of a wide variety of materials known in the art to facilitate the bonding

between such layers. Typically, the adhesion promoter layer 114 comprises a silane

5        compound. In alternative embodiments, protein or like molecules in the analyte sensing

layer 110 can be sufficiently crosslinked or otherwise prepared to allow the analyte

modulating membrane layer 112 to be disposed in direct contact with the analyte sensing

layer 110 in the absence of an adhesion promoter layer 114.


10    **C.        TYPICAL ANALYTE SENSOR CONSTITUENTS**

The following disclosure provides examples of typical elements/constituents

used in the sensors of the invention. While these elements can be described as discreet

units (e.g. layers), those of skill in the art understand that sensors can be designed to

contain elements having a combination of some or all of the material properties and/or

15    functions of the elements/constituents discussed below (e.g. an element that serves both

as a supporting base constituent and/or a conductive constituent and/or a matrix for the

analyte sensing constituent and which further functions as an electrode in the sensor).


BASE CONSTITUENT

20        Sensors of the invention typically include a base constituent (see, e.g. element 102

in Figure 2). The term "base constituent" is used herein according to art accepted

terminology and refers to the constituent in the apparatus that typically provides a

supporting matrix for the plurality of constituents that are stacked on top of one another

and comprise the functioning sensor. In one form, the base constituent comprises a thin

25    film sheet of insulative (e.g. electrically insulative and/or water impermeable) material.

This base constituent can be made of a wide variety of materials having desirable

qualities such as water impermeability and hermeticity. Some materials include metallic

ceramic and polymeric substrates or the like. In certain embodiments, the base

constituent and/or the conductive constituent can be constructed to produce electrodes

30    having a configuration where the electrochemically reactive surface area of an electrode

is disposed on the geometric feature so that the electrochemically reactive surface area is greater than if it was disposed on a flat surface.

The base constituent may be self-supporting or further supported by another material as is known in the art. In one embodiment of the sensor configuration shown in

5    Figure 2, the base constituent 102 comprises a ceramic. In an illustrative embodiment, the ceramic base comprises a composition that is predominantly $Al_2O_3$ (e.g. 96%). The use of alumina as an insulating base constituent for use with implantable devices is disclosed in U.S. Pat. Nos. 4,940,858, 4,678,868 and 6,472,122 which are incorporated herein by reference. The base constituents of the invention can further include other

10   elements known in the art, for example hermetical vias (see, e.g. WO 03/023388). Depending upon the specific sensor design, the base constituent can be relatively thick constituent (e.g. thicker than 25 microns). Alternatively, one can utilize a nonconductive ceramic, such as alumina, in thin constituents, e.g., less than about 25 microns.

Embodiments of invention disclosed herein provide individual elements and

15   sensors which exhibit a combination of the independent advantages found in each of the two sensor classes disclosed above. For example a first embodiment of the invention immobilizes an enzyme onto a thick (1-1,000 micron), porous substrate which functions as an electrode in the sensor. In this context, the porous electrode is designed to exhibit an increased surface area, for example by constructing it from a lattice of equal-sized

20   adjoining spheres. In one illustrative embodiment, glucose oxidase is immobilized on a thick (1-1,000 micron), porous metallic substrate that is manufactured from a lattice of equal-sized adjoining spheres and which function as a hydrogen peroxide-consuming electrode.

The advantage of such a thick, porous electrode matrices relative to thin, flat

·25   electrode matrices is demonstrated using Equation (1):

$$\frac{A_{avail}}{A_{proj}} = \frac{3L\,(1-\epsilon)\phi}{R} \tag{1}$$

where the thick, porous electrode is modeled as a lattice of equal-sized adjoining spheres,

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while the thin electrode is modeled as a two-dimensional surface. The surface area available for enzyme or protein immobilization is $A_{avail}$, while the projected area of the electrode is $A_{proj}$. The porosity and thickness of the electrode are $L$ and $\varepsilon$, respectively. The spheres making-up the thick electrode are of radius $R$, while the fraction of the spheres' surface area available for enzyme or protein immobilization is $\phi$. For example, a porous electrode with $L = 25$ μm, $R = 1$ μm, $\varepsilon = 0.5$, and $\phi = 0.5$ would possess more than 18 times the surface area for enzyme immobilization as compared to a thin electrode with same projected area.

The porosity range of the such as the porous electrode matrices discussed above is typically 5-99%, 10-99%, 20-99%, 30-99%, 40-99%, 50-99% or 60-99%. The porosity of matrices can be evaluated using any one of a variety of methods known in the art. In certain contexts for example, artisans may wish to examine porosity of a matrix via mercury porosimetry (see, e.g. U.S. Patent No. 5,609,839), liquid intrusion porosimetry (see, e.g. U.S. Patent No. 4,660,412), gas porosimetry (see, e.g. Dombrowski et al., Langmuir 16: 5041-5050 (2000) and Lastoskie et al., Journal of Physical Chemistry 97: 4786-4796 (1993)), or by cyclic voltametry and/or methods which employ size exclusion chromatography using marker molecules of various sizes and molecular weights (e.g. acetone, various globular proteins, blue dextran etc.).

The terms nano-porous, micro-porous and macro-porous are used when discussing certain embodiments of the porous matrices that are disclosed herein. For example, platinum-black is commonly used to increase the electrochemically effective surface area of a working electrode. Standard platinum-black electrodes have a great deal of porosity, with the pores being sized so that only very small molecules like $H_2$, $O_2$, and $H_2O$ can get inside them. Platinum-black electrodes having this characteristic are termed nano-porous. Such nano-pores have a size range that permits small molecules like $H_2$, $O_2$, and $H_2O$ the get inside them, but prevents larger molecules like GOx from getting inside. In certain embodiments of the invention, the electrodes used in the sensors have both nano- and micro-porosity. Micro-pores are characterized in that they are large enough to allow molecules such as GOx to be immobilized inside of them, but are small enough so that any molecule of GOx is relatively close (less than about 0.1, 1, or 2

microns) to the surface of the working electrode. Electrodes having this micro-porosity exhibit a number of desirable characteristics. For example, as the working electrode of an $H_2O_2$-based sensor consumes $H_2O_2$ and $H_2O_2$ is believed to contribute to the deactivation GOx over time, micro-porous electrodes that allow the placement of

5     immobilized GOx in close proximity to an $H_2O_2$-consuming electrode will increase the lifetime of GOx in the sensor.

        In another embodiment of the invention disclosed herein the hydrogel typically utilized in a variety of analyte sensors is replaced with an essentially rigid, non-swelling porous enzyme-polymer matrix. In this embodiment, bio-sensing enzymes can be stably

10    immobilized via covalent bonding to a rigid, macroporous polymer that has optionally been molded into a specified shape. In this context, molded continuous rods of macroporous polymers have been developed for use as chromatographic separation media (see, e.g. US 5,453,185 and WO 93/07945). Suitable polymers are essentially incompressible and do not change their overall size in response to changes in their

15    solvating environment. Moreover, adjustments to the polymerization conditions can be used to control the morphology of the pores. Hence, highly porous (50-70%) polymers can be created that possess significant volume fractions of pores in the ranges of 1-100 nm and 100-3,000 nm (i.e. 20% and 80%, respectively). Polymers with this type of pore structure possess a very high specific surface area (i.e. 185 $m^2/g$), and are expected to

20    allow for high enzyme immobilization densities (1-100 mg/mL).

        Various methods and compositions for making and using the above-noted porous matrices as well as analyte sensors which incorporate such matrices are further described herein.


25    CONDUCTIVE CONSTITUENT

        The electrochemical sensors of the invention typically include a conductive constituent disposed upon the base constituent that includes at least one electrode for contacting an analyte or its byproduct (e.g. oxygen and/or hydrogen peroxide) to be assayed (see, e.g. element 104 in Figure 2). The term "conductive constituent" is used

30    herein according to art accepted terminology and refers to electrically conductive sensor

elements such as electrodes which are capable of measuring and a detectable signal and conducting this to a detection apparatus. An illustrative example of this is a conductive constituent that can measure an increase or decrease in current in response to exposure to a stimuli such as the change in the concentration of an analyte or its byproduct as

5       compared to a reference electrode that does not experience the change in the concentration of the analyte, a coreactant (e.g. oxygen) used when the analyte interacts with a composition (e.g. the enzyme glucose oxidase) present in analyte sensing constituent 110 or a reaction product of this interaction (e.g. hydrogen peroxide). Illustrative examples of such elements include electrodes which are capable of producing

10      a variable detectable signals in the presence of variable concentrations of molecules such as hydrogen peroxide or oxygen. Typically one of these electrodes in the conductive constituent is a working electrode, which can be made from non-corroding metal or carbon. A carbon working electrode may be vitreous or graphitic and can be made from a solid or a paste. A metallic working electrode may be made from platinum group

15      metals, including palladium or gold, or a non-corroding metallically conducting oxide, such as ruthenium dioxide. Alternatively the electrode may comprise a silver/silver chloride electrode composition. The working electrode may be a wire or a thin conducting film applied to a substrate, for example, by coating or printing. Typically, only a portion of the surface of the metallic or carbon conductor is in electrolytic contact

20      with the analyte-containing solution. This portion is called the working surface of the electrode. The remaining surface of the electrode is typically isolated from the solution by an electrically insulating cover constituent 106. Examples of useful materials for generating this protective cover constituent 106 include polymers such as polyimides, polytetrafluoroethylene, polyhexafluoropropylene and silicones such as polysiloxanes.

25              In addition to the working electrode, the analyte sensors of the invention typically include a reference electrode or a combined reference and counter electrode (also termed a quasi-reference electrode or a counter/reference electrode). If the sensor does not have a counter/reference electrode then it may include a separate counter electrode, which may be made from the same or different materials as the working

30      electrode. Typical sensors of the present invention have one or more working electrodes

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and one or more counter, reference, and/or counter/reference electrodes. One

embodiment of the sensor of the present invention has two, three or four or more

working electrodes. These working electrodes in the sensor may be integrally connected

or they may be kept separate.

5          Typically, for in vivo use the analyte sensors of the present invention are

implanted subcutaneously in the skin of a mammal for direct contact with the body fluids

of the mammal, such as blood. Alternatively the sensors can be implanted into other

regions within the body of a mammal such as in the intraperotineal space. When

multiple working electrodes are used, they may be implanted together or at different

10         positions in the body. The counter, reference, and/or counter/reference electrodes may

also be implanted either proximate to the working electrode(s) or at other positions

within the body of the mammal.


INTERFERENCE REJECTION CONSTITUENT

15         The electrochemical sensors of the invention optionally include an interference

rejection constituent disposed between the surface of the electrode and the environment

to be assayed. In particular, certain sensor embodiments rely on the oxidation and/or

reduction of hydrogen peroxide generated by enzymatic reactions on the surface of a

working electrode at a constant potential applied. Because amperometric detection

20         based on direct oxidation of hydrogen peroxide requires a relatively high oxidation

potential, sensors employing this detection scheme may suffer interference from

oxidizable species that are present in biological fluids such as ascorbic acid, uric acid and

acetaminophen. In this context, the term "interference rejection constituent" is used

herein according to art accepted terminology and refers to a coating or membrane in the

25         sensor that functions to inhibit spurious signals generated by such oxidizable species

which interfere with the detection of the signal generated by the analyte to be sensed.

Examples of interference rejection constituents include one or more layers or coatings of

compounds such as hydrophilic polyurethanes, cellulose acetate (including cellulose

acetate incorporating agents such as poly(ethylene glycol), polyethersulfones, polytetra-

30         fluoroethylenes, the perfluoronated ionomer Nafion™, polyphenylenediamine, epoxy

and the like. Illustrative discussions of such interference rejection constituents are found

for example in Ward et al., Biosensors and Bioelectronics 17 (2002) 181-189 and Choi et

al., Analytical Chimica Acta 461 (2002) 251-260 which are incorporated herein by

reference.

5

ANALYTE SENSING CONSTITUENT

　　　　The electrochemical sensors of the invention include an analyte sensing

constituent disposed on the electrodes of the sensor (see, e.g. element 110 in Figure 2).

The term "analyte sensing constituent" is used herein according to art accepted

10   terminology and refers to a constituent comprising a material that is capable of

recognizing or reacting with an analyte whose presence is to be detected by the analyte

sensor apparatus. Typically this material in the analyte sensing constituent produces a

detectable signal after interacting with the analyte to be sensed, typically via the

electrodes of the conductive constituent. In this regard the analyte sensing constituent

15   and the electrodes of the conductive constituent work in combination to produce the

electrical signal that is read by an apparatus associated with the analyte sensor. Typically,

the analyte sensing constituent comprises an enzyme capable of reacting with and/or

producing a molecule whose change in concentration can be measured by measuring the

change in the current at an electrode of the conductive constituent (e.g. oxygen and/or

20   hydrogen peroxide), for example the enzyme glucose oxidase. An enzyme capable of

producing a molecule such as hydrogen peroxide can be disposed on the electrodes

according to a number of processes known in the art. The analyte sensing constituent

can coat all or a portion of the various electrodes of the sensor. In this context, the

analyte sensing constituent may coat the electrodes to an equivalent degree. Alternatively

25   the analyte sensing constituent may coat different electrodes to different degrees, with

for example the coated surface of the working electrode being larger than the coated

surface of the counter and/or reference electrode.

　　　　Typical sensor embodiments of this element of the invention utilize an enzyme

(e.g. glucose oxidase) that has been combined with a second protein (e.g. albumin) in a

30   fixed ratio (e.g. one that is typically optimized for glucose oxidase stabilizing properties)

and then applied on the surface of an electrode to form a thin enzyme constituent. In a typical embodiment, the analyte sensing constituent comprises a GOx and HSA mixture. A typical embodiment of an analyte sensing constituent having GOx, the GOx reacts with glucose present in the sensing environment (e.g. the body of a mammal) and

5  generates hydrogen peroxide according to the reaction shown in Figure 1, wherein the hydrogen peroxide so generated is anodically detected at the working electrode in the conductive constituent. As discussed for example in U.S. Patent Application Serial Number 10/273,767 (incorporated herein by reference) extremely thin sensor chemistry constituents are typical and can be applied to the surface of the electrode matrix by

10 processes known in the art such as spin coating. In an illustrative embodiment, a glucose oxidase/albumin is prepared in a physiological solution (e.g., phosphate buffered saline at neutral pH) with the albumin being present in an range of about .5%-10% by weight. Optionally the stabilized glucose oxidase constituent that is formed on the analyte sensing constituent is very thin as compared to those previously described in the art, for

15 example less than 2, 1, 0.5, 0.25 or 0.1 microns in thickness. One illustrative embodiment of the invention utilizes a stabilized glucose oxidase constituent for coating the surface of an electrode wherein the glucose oxidase is mixed with a carrier protein in a fixed ratio within the constituent, and the glucose oxidase and the carrier protein are distributed in a substantially uniform manner throughout the constituent. Typically the

20 constituent is less than 2 microns in thickness. For purposes of clarity, it should be noted that this may not apply to certain embodiments of the invention where the analyte sensing constituent is disposed on a porous electrode. For example, in a porous electrode that is 100 microns thick, with 3 micron size pores that are filled with Gox, an enzyme layer can be greater 2 microns.

25       Surprisingly, sensors having these extremely thin analyte sensing constituents have material properties that exceed those of sensors having thicker coatings including enhanced longevity, linearity, regularity as well as improved signal to noise ratios. While not being bound by a specific scientific theory, it is believed that sensors having extremely thin analyte sensing constituents have surprisingly enhanced characteristics as

30 compared to those of thicker constituents because in thicker enzyme constituents only a

fraction of the reactive enzyme within the constituent is able to access the analyte to be sensed. In sensors utilizing glucose oxidase, the thick coatings produced by electrodeposition may hinder the ability of hydrogen peroxide generated at the reactive interface of a thick enzyme constituent to contact the sensor surface and thereby

5      generate a signal.

As noted above, the enzyme and the second protein are typically treated to form a crosslinked matrix (e.g. by adding a cross-linking agent to the protein mixture). As is known in the art, crosslinking conditions may be manipulated to modulate factors such as the retained biological activity of the enzyme, its mechanical and/or operational

10     stability. Illustrative crosslinking procedures are described in U.S. Patent Application Serial Number 10/335,506 and PCT publication WO 03/035891 which are incorporated herein by reference. For example, an amine cross-linking reagent, such as, but not limited to, glutaraldehyde, can be added to the protein mixture. The addition of a cross-linking reagent to the protein mixture creates a protein paste. The concentration of the

15     cross-linking reagent to be added may vary according to the concentration of the protein mixture. While glutaraldehyde is an illustrative crosslinking reagent, other cross-linking reagents may also be used or may be used in place of glutaraldehyde, including, but not limited to, an amine reactive, homofunctional, cross-linking reagent such as Disuccinimidyl Suberate (DSS). Another example is 1-Ethyl-3 (3-Dimethylaminopropyl)

20     Carbodiimide (EDC), which is a zero-length cross-linker. EDC forms an amide bond between carboxylic acid and amine groups. Other suitable cross-linkers also may be used, as will be evident to those skilled in the art.

The GOx and/or carrier protein concentration may vary for different embodiments of the invention. For example, the GOx concentration may be within the

25     range of approximately 50 mg/ml (approximately 10,000 U/ml) to approximately 700 mg/ml (approximately 150,000 U/ml). Typically the GOx concentration is about 115 mg/ml (approximately 22,000 U/ml). In such embodiments, the HSA concentration may vary between about 0.5%-30% (w/v), depending on the GOx concentration. Typically the HSA concentration is about 1-10% w/v, and most typically is about 5%

30     w/v. In alternative embodiments of the invention, collagen or BSA or other structural

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proteins used in these contexts can be used instead of or in addition to HSA. Although GOx is discussed as an illustrative enzyme in the analyte sensing constituent, other proteins and/or enzymes may also be used or may be used in place of GOx, including, but not limited to glucose dehydrogenase or hexokinase, hexose oxidase, lactate oxidase, and the like. Other proteins and/or enzymes may also be used, as will be evident to those skilled in the art. Moreover, although HSA is employed in the example embodiment, other structural proteins, such as BSA, collagens or the like, could be used instead of or in addition to HSA.

For embodiments employing enzymes other than GOx, concentrations other than those discussed herein may be utilized. For example, depending on the enzyme employed, concentrations ranging from approximately 10% weight per weight to 70% weight per weight may be suitable. The concentration may be varied not only depending on the particular enzyme being employed, but also depending on the desired properties of the resulting protein matrix. For example, a certain concentration may be utilized if the protein matrix is to be used in a diagnostic capacity while a different concentration may be utilized if certain structural properties are desired. Those skilled in the art will understand that the concentration utilized may be varied through experimentation to determine which concentration (and of which enzyme or protein) may yield the desired result.

As noted above, in some embodiments of the invention, the analyte sensing constituent includes a composition (e.g. glucose oxidase) capable of producing a signal (e.g. a change in oxygen and/or hydrogen peroxide concentrations) that can be sensed by the electrically conductive elements (e.g. electrodes which sense changes in oxygen and/or hydrogen peroxide concentrations). However, other useful analyte sensing constituents can be formed from any composition that is capable of producing a detectable signal that can be sensed by the electrically conductive elements after interacting with a target analyte whose presence is to be detected. In some embodiments, the composition comprises an enzyme that modulates hydrogen peroxide concentrations upon reaction with an analyte to be sensed. Alternatively, the composition comprises an enzyme that modulates oxygen concentrations upon reaction

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with an analyte to be sensed. In this context, a wide variety of enzymes that either use or produce hydrogen peroxide and/or oxygen in a reaction with a physiological analyte are known in the art and these enzymes can be readily incorporated into the analyte sensing constituent composition. A variety of other enzymes known in the art can produce

5    and/or utilize compounds whose modulation can be detected by electrically conductive elements such as the electrodes that are incorporated into the sensor designs described herein. Such enzymes include for example, enzymes specifically described in Table 1, pages 15-29 and/or Table 18, pages 111-112 of Protein Immobilization: Fundamentals and Applications (Bioprocess Technology, Vol 14) by Richard F. Taylor (Editor)

10   Publisher: Marcel Dekker; (January 7, 1991) the entire contents of which are incorporated herein by reference.

Other useful analyte sensing constituents can be formed to include antibodies whose interaction with a target analyte is capable of producing a detectable signal that can be sensed by the electrically conductive elements after interacting with the target

15   analyte whose presence is to be detected. For example U.S. Patent No. 5,427,912 (which is incorporated herein by reference) describes an antibody-based apparatus for electrochemically determining the concentration of an analyte in a sample. In this device, a mixture is formed which includes the sample to be tested, an enzyme-acceptor polypeptide, an enzyme-donor polypeptide linked to an analyte analog (enzyme-donor

20   polypeptide conjugate), a labeled substrate, and an antibody specific for the analyte to be measured. The analyte and the enzyme-donor polypeptide conjugate competitively bind to the antibody. When the enzyme-donor polypeptide conjugate is not bound to antibody, it will spontaneously combine with the enzyme acceptor polypeptide to form an active enzyme complex. The active enzyme then hydrolyzes the labeled substrate,

25   resulting in the generation of an electroactive label, which can then be oxidized at the surface of an electrode. A current resulting from the oxidation of the electroactive compound can be measured and correlated to the concentration of the analyte in the sample. U.S. Patent No. 5,149,630 (which is incorporated herein by reference) describes an electrochemical specific binding assay of a ligand (e.g., antigen, hapten or antibody)

30   wherein at least one of the components is enzyme-labelled, and which includes the step

of determining the extent to which the transfer of electrons between the enzyme
substrate and an electrode, associated with the substrate reaction, is perturbed by
complex formation or by displacement of any ligand complex relative to unbound
enzyme-labelled component. The electron transfer is aided by electron-transfer

5     mediators which can accept electrons from the enzyme and donate them to the electrode
or vice versa (e.g. ferrocene) or by electron-transfer promoters which retain the enzyme
in close proximity with the electrode without themselves taking up a formal charge. U.S.
Patent No. 5,147,781 (which is incorporated herein by reference) describes an assay for
the determination of the enzyme lactate dehydrogenase-5 (LDH5) and to a biosensor for

10    such quantitative determination. The assay is based on the interaction of this enzyme
with the substrate lactic acid and nicotine-amine adenine dinucleotide (NAD) to yield
pyruvic acid and the reduction product of NAD. Anti-LDH5 antibody is bound to a
suitable glassy carbon electrode; this is contacted with the substrate containing LDH5,
rinsed, inserted into a NAD solution, connected to an amperometric system, current

15    changes are measured in the presence of differing concentrations of lactic acid, which are
indicative of the quantity of LDH-5. U.S. Patent No. 6,410,251 (which is incorporated
herein by reference) describes an apparatus and method for detecting or assaying one
constituting member in a specific binding pair; for example, the antigen in an
antigen/antibody pair, by utilizing specific binding such as binding between an antigen

20    and an antibody, together with redox reaction for detecting a label, wherein an oxygen
micro-electrode with a sensing surface area is used. In addition, U.S. Patent No.
4,402,819 (which is incorporated herein by reference) describes an antibody-selective
potentiometric electrode for the quantitative determination of antibodies (as the analyte)
in dilute liquid serum samples employing an insoluble membrane incorporating an

25    antigen having bonded thereto an ion carrier effecting the permeability of preselected
cations therein, which permeability is a function of specific antibody concentrations in
analysis, and the corresponding method of analysis. For related disclosures, see also
U.S. Patent Nos. 6,703,210, 5,981,203, 5,705,399 and 4,894,253, the contents of which
are incorporated herein by reference.

30            In addition to enzymes and antibodies, other exemplary materials for use in the

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analyte sensing constituents of the sensors disclosed herein include polymers that bind specific types of cells or cell components (e.g. polypeptides, carbohydrates and the like); single-strand DNA; antigens and the like. The detectable signal can be, for example, an optically detectable change, such as a color change or a visible accumulation of the

5        desired analyte (e.g., cells). Sensing elements can also be formed from materials that are essentially non-reactive (i.e., controls). The foregoing alternative sensor elements are beneficially included, for example, in sensors for use in cell-sorting assays and assays for the presence of pathogenic organisms, such as viruses (HIV, hepatitis-C, etc.), bacteria, protozoa and the like.

10        Also contemplated are analyte sensors that measure an analyte that is present in the external environment and that can in itself produce a measurable change in current at an electrode. In sensors measuring such analytes, the analyte sensing constituent can be optional.


15   PROTEIN CONSTITUENT

The electrochemical sensors of the invention optionally include a protein constituent disposed between the analyte sensing constituent and the analyte modulating constituent (see, e.g. element 116 in Figure 2). The term "protein constituent" is used herein according to art accepted terminology and refers to constituent containing a

20        carrier protein or the like that is selected for compatibility with the analyte sensing constituent and/or the analyte modulating constituent. In typical embodiments, the protein constituent comprises an albumin such as human serum albumin. The HSA concentration may vary between about 0.5%-30% (w/v). Typically the HSA concentration is about 1-10% w/v, and most typically is about 5% w/v. In alternative

25        embodiments of the invention, collagen or BSA or other structural proteins used in these contexts can be used instead of or in addition to HSA. This constituent is typically crosslinked on the analyte sensing constituent according to art accepted protocols.


ADHESION PROMOTING CONSTITUENT

30        The electrochemical sensors of the invention can include one or more adhesion

promoting (AP) constituents (see, e.g. element 114 in Figure 2). The term "adhesion promoting constituent" is used herein according to art accepted terminology and refers to a constituent that includes materials selected for their ability to promote adhesion between adjoining constituents in the sensor. Typically, the adhesion promoting

5      constituent is disposed between the analyte sensing constituent and the analyte modulating constituent. Typically, the adhesion promoting constituent is disposed between the optional protein constituent and the analyte modulating constituent. The adhesion promoter constituent can be made from any one of a wide variety of materials known in the art to facilitate the bonding between such constituents and can be applied

10     by any one of a wide variety of methods known in the art. Typically, the adhesion promoter constituent comprises a silane compound such as γ-aminopropyltrimethoxysilane.

The use of silane coupling reagents, especially those of the formula R'Si(OR)₃ in which R' is typically an aliphatic group with a terminal amine and R is a lower alkyl

15     group, to promote adhesion is known in the art (see, e.g. U.S. Patent No. 5,212,050 which is incorporated herein by reference). For example, chemically modified electrodes in which a silane such as γ-aminopropyltriethoxysilane and glutaraldehyde were used in a step-wise process to attach and to co-crosslink bovine serum albumin (BSA) and glucose oxidase (GOx) to the electrode surface are well known in the art (see, e.g. Yao, T.

20     Analytica Chim. Acta 1983, 148, 27-33).

In certain embodiments of the invention, the adhesion promoting constituent further comprises one or more compounds that can also be present in an adjacent constituent such as the polydimethyl siloxane (PDMS) compounds that serves to limit the diffusion of analytes such as glucose through the analyte modulating constituent. In

25     illustrative embodiments the formulation comprises 0.5-20% PDMS, typically 5-15% PDMS, and most typically 10% PDMS. In certain embodiments of the invention, the adhesion promoting constituent includes an agent selected for its ability to crosslink a siloxane moiety present in a proximal constituent such as the analyte modulating constituent. In closely related embodiments of the invention, the adhesion promoting

30     constituent includes an agent selected for its ability to crosslink an amine or carboxyl

moiety of a protein present in a proximal constituent such a the analyte sensing constituent and/or the protein constituent.

ANALYTE MODULATING CONSTITUENT

5        The electrochemical sensors of the invention include an analyte modulating constituent disposed on the sensor (see, e.g. element 112 in Figure 2). The term "analyte modulating constituent" is used herein according to art accepted terminology and refers to a constituent that typically forms a membrane on the sensor that operates to modulate the diffusion of one or more analytes, such as glucose, through the constituent. In

10  certain embodiments of the invention, the analyte modulating constituent is an analyte-limiting membrane which operates to prevent or restrict the diffusion of one or more analytes, such as glucose, through the constituents. In other embodiments of the invention, the analyte-modulating constituent operates to facilitate the diffusion of one or more analytes, through the constituents. Optionally such analyte modulating

15  constituents can be formed to prevent or restrict the diffusion of one type of molecule through the constituent (e.g. glucose), while at the same time allowing or even facilitating the diffusion of other types of molecules through the constituent (e.g. $O_2$).

       With respect to glucose sensors, in known enzyme electrodes, glucose and oxygen from blood, as well as some interferants, such as ascorbic acid and uric acid,

20  diffuse through a primary membrane of the sensor. As the glucose, oxygen and interferants reach the analyte sensing constituent, an enzyme, such as glucose oxidase, catalyzes the conversion of glucose to hydrogen peroxide and gluconolactone. The hydrogen peroxide may diffuse back through the analyte modulating constituent, or it may diffuse to an electrode where it can be reacted to form oxygen and a proton to

25  produce a current that is proportional to the glucose concentration. The sensor membrane assembly serves several functions, including selectively allowing the passage of glucose therethrough. In this context, an illustrative analyte modulating constituent is a semi-permeable membrane which permits passage of water, oxygen and at least one selective analyte and which has the ability to absorb water, the membrane having a water

30  soluble, hydrophilic polymer.

A variety of illustrative analyte modulating compositions are known in the art and are described for example in U.S. Patent Nos. 6,319,540, 5,882,494, 5,786,439 5,777,060, 5,771,868 and 5,391,250, the disclosures of each being incorporated herein by reference. The hydrogels described therein are particularly useful with a variety of implantable

5      devices for which it is advantageous to provide a surrounding water constituent. In some embodiments of the invention, the analyte modulating composition includes PDMS. In certain embodiments of the invention, the analyte modulating constituent includes an agent selected for its ability to crosslink a siloxane moiety present in a proximal constituent. In closely related embodiments of the invention, the adhesion

10     promoting constituent includes an agent selected for its ability to crosslink an amine or carboxyl moiety of a protein present in a proximal constituent.


COVER CONSTITUENT

The electrochemical sensors of the invention include one or more cover

15     constituents which are typically electrically insulating protective constituents (see, e.g. element 106 in Figure 2). Typically, such cover constituents are disposed on at least a portion of the analyte modulating constituent. Acceptable polymer coatings for use as the insulating protective cover constituent can include, but are not limited to, non-toxic biocompatible polymers such as silicone compounds, polyimides, biocompatible solder

20     masks, epoxy acrylate copolymers, or the like. Further, these coatings can be photo-imageable to facilitate photolithographic forming of apertures through to the conductive constituent. A typical cover constituent comprises spun on silicone. As is known in the art, this constituent can be a commercially available RTV (room temperature vulcanized) silicone composition. A typical chemistry in this context is polydimethyl siloxane

25     (acetoxy based).

Various illustrative embodiments of the invention and their characteristics are discussed in detail in the following sections.


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## C.     ILLUSTRATIVE EMBODIMENTS OF ANALYTE SENSOR APPARATUS AND ASSOCIATED CHARACTERISTICS

5          The analyte sensor apparatus disclosed herein has a number of embodiments. A general embodiment of the invention is an analyte sensor apparatus for implantation within a mammal. While the analyte sensors are typically designed to be implantable within the body of a mammal, the sensors are not limited to any particular environment can instead be used in a wide variety of contexts, for example for the analysis of most

10       liquid samples including biological fluids such as whole-blood, lymph, plasma, serum, saliva, urine, stool, perspiration, mucus, tears, cerebrospinal fluid, nasal secretion, cervical or vaginal secretion, semen, pleural fluid, amniotic fluid, peritoneal fluid, middle ear fluid, joint fluid, gastric aspirate or the like. In addition, solid or desiccated samples may be dissolved in an appropriate solvent to provide a liquid mixture suitable for analysis.

15       As noted above, the sensor embodiments disclosed herein can be used to sense analytes of interest in one or more physiological environments. In certain embodiments for example, the sensor can be in direct contact with interstitial fluids as typically occurs with subcutaneous sensors. The sensors of the present invention may also be part of a skin surface system where interstitial glucose is extracted through the skin and brought

20       into contact with the sensor (see, e.g. 6,155,992 and 6,706,159 which are incorporated herein by reference). In other embodiments, the sensor can be in contact with blood as typically occurs for example with intravenous sensors. The sensor embodiments of the invention further include those adapted for use in a variety of contexts. In certain embodiments for example, the sensor can be designed for use in mobile contexts, such

25       as those employed by ambulatory users. Alternatively, the sensor can be designed for use in stationary contexts such as those adapted for use in clinical settings. Such sensor embodiments include, for example, those used to monitor one or more analytes present in one or more physiological environments in a hospitalized patient.

Sensors of the invention can also be incorporated in to a wide variety of medical

30       systems known in the art. Sensors of the invention can be used, for example, in a closed loop infusion systems designed to control the rate that medication is infused into the

body of a user. Such a closed loop infusion system can include a sensor and an associated meter which generates an input to a controller which in turn operates a delivery system (e.g. one that calculates a dose to be delivered by a medication infusion pump). In such contexts, the meter associated with the sensor may also transmit

5      commands to, and be used to remotely control, the delivery system. Typically, the sensor is a subcutaneous sensor in contact with interstitial fluid to monitor the glucose concentration in the body of the user, and the liquid infused by the delivery system into the body of the user includes insulin. Illustrative systems are disclosed for example in U. S. Patent Nos. 6,558,351 and 6,551,276; PCT Application Nos. US99/21703 and

10     US99/22993; as well as WO 2004/008956 and WO 2004/009161, all of which are incorporated herein by reference.

            Certain embodiments of the invention measure peroxide and have the advantageous characteristic of being suited for implantation in a variety of sites in the mammal including regions of subcutaneous implantation and intravenous implantation as

15     well as implantation into a variety of non-vascular regions. A peroxide sensor design that allows implantation into non-vascular regions has advantages over certain sensor apparatus designs that measure oxygen due to the problems with oxygen noise that can occur in oxygen sensors implanted into non-vascular regions. For example, in such implanted oxygen sensor apparatus designs, oxygen noise at the reference sensor can

20     compromise the signal to noise ratio which consequently perturbs their ability to obtain stable glucose readings in this environment. The peroxide sensors of the invention therefore overcome the difficulties observed with such oxygen sensors in non-vascular regions.

            Certain peroxide sensor embodiments of the invention further include

25     advantageous long term or "permanent" sensors which are suitable for implantation in a mammal for a time period of greater than 30 days. In particular, as is known in the art (see, e.g. ISO 10993, Biological Evaluation of Medical Devices) medical devices such as the sensors described herein can be categorized into three groups based on implant duration: (1) "Limited" (< 24 hours), (2) "Prolonged" (24 hours - 30 days), and (3)

30     "Permanent" (> 30 days). In some embodiments of the invention, the design of the

peroxide sensor of the invention allows for a "Permanent" implantation according to this categorization, i.e. > 30 days. In related embodiments of the invention, the highly stable design of the peroxide sensor of the invention allows for an implanted sensor to continue to function in this regard for 2, 3, 4, 5, 6 or 12 or more months.

5        In general, the analyte sensor apparatus structure comprises a base layer and a conductive layer disposed upon the base layer (e.g. a porous matrix) and functions as one or more electrodes. For example, the conductive layer can include a working electrode, a reference electrode and/or a counter electrode. These electrodes can be spaced in proximity, or alternatively are spaced distally, according to the specific design. The
10      sensor apparatus design is such that certain electrodes (e.g. the working electrode) can be exposed to the solution containing the analyte to be sensed (e.g. via an aperture) in the sensor apparatus. The sensor apparatus design is such that certain electrodes (e.g. the reference electrode) are not exposed to the solution containing the analyte to be sensed in the sensor apparatus.

15      One embodiment of the invention is a composition for use in biosensors. Such compositions are typically designed to implantable within a mammal and comprise a porous matrix having a surface coated with an immobilized enzyme, for example glucose oxidase, glucose dehydrogenase, lactate oxidase, hexokinase or lactate dehydrogenase. Typically the porous matrix coated with an immobilized enzyme is capable of acting as
20      an electrode in an electrochemical sensor. Optionally the electrode in the electrochemical sensor consumes hydrogen peroxide.

The porous matrices used in various embodiments of the biosensors of the invention can be generated from a variety of materials and can be adapted to a variety of compositional configurations. In some embodiments of the invention, the porous matrix
25      comprises a ceramic material and/or a metal and/or a macroporous polymer. Optionally the porous matrix comprises a lattice of particles. Typically the particles are spherical. In typical embodiments of the invention, porous matrix has a surface area that is at least 2, 4, 6, 8, 10, 12, 14, 16 or 18 times the surface area of a non-porous matrix of same dimensions. In certain embodiments of the invention, the porous matrix is at least 1, 10,
30      100, or 1000 microns thick. In certain embodiments of the invention, the porosity range

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of the porous matrix is optionally about 5-99.9% and typically is about 40-99%. The porosity of these matrices can be measured by one of the protocols typically used in the art such as mercury or gas porosimetry, size-exclusion chromatography using marker molecules of various sizes and molecular weights (e.g. acetone, various globular proteins

5      of a defined size, blue dextran), and cyclic voltammetry.

A related embodiment of the invention is an analyte sensor apparatus for implantation within a mammal which includes a porous matrix having a surface coated with an immobilized enzyme, for example glucose oxidase. In one embodiment of this sensor design, the porous matrix comprises a working electrode; and the immobilized

10     enzyme is disposed within an analyte sensing layer disposed on the working electrode, such that the analyte sensing layer detectably alters the electrical current at the working electrode in the conductive layer in the presence of an analyte. Typically the sensor further comprises an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the diffusion of the analyte

15     therethrough. Typically, the sensor further comprises an adhesion promoting layer disposed on the analyte sensing layer, wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer. Optionally the sensor further comprises a protein layer disposed between the analyte sensing layer and the analyte modulating layer. Typically

20     the sensor further comprises a cover layer disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture that exposes at least a portion of the analyte modulating layer to a solution comprising the analyte to be sensed.

A related embodiment of the invention is a method of making a sensor apparatus

25     for implantation within a mammal comprising the steps of providing a layer comprising a porous matrix, forming an analyte sensing layer on the porous matrix, wherein the analyte sensing layer includes an enzyme such as glucose oxidase that can alter the electrical current at the surface of the porous matrix in the presence of an analyte so that the porous matrix having the analyte sensing layer formed thereon functions as an

30     electrode. Such methods further include the steps of optionally forming a protein layer

on the analyte sensing layer, forming an adhesion promoting layer on the analyte sensing layer or the optional protein layer, forming an analyte modulating layer disposed on the adhesion promoting layer, wherein the analyte modulating layer includes a composition that modulates the diffusion of the analyte therethrough; and forming a cover layer

5       disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer.

Another embodiment of the invention is a method of sensing an analyte within the body of a mammal, the method comprising implanting an analyte sensor into the mammal, the analyte sensor comprising a porous matrix having an analyte sensing layer

10      disposed thereon, wherein the analyte sensing layer detectably alters the electrical current at the surface of the porous matrix in the presence of an analyte so that the porous matrix having the analyte sensing layer formed thereon functions as an electrode, an optional protein layer disposed on the analyte sensing layer, an adhesion promoting layer disposed on the analyte sensing layer or the optional protein layer, wherein the adhesion

15      promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer, and an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the diffusion of the analyte therethrough, a cover layer disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at

20      least a portion of the analyte modulating layer; and sensing an alteration in electrical current and correlating the alteration in current with the presence of the analyte, so that the analyte is sensed.

Yet another embodiment of the invention is a method of immobilizing a protein on a rigid macroporous polymer comprising the steps of: combining the protein with the

25      rigid macroporous polymer having functional moieties capable of crosslinking to a protein; and then adding a crosslinking agent capable of immobilizing the protein on the rigid macroporous polymer by crosslinking the functional moieties of the protein with the functional moieties of the rigid macroporous polymer so that the protein is immobilized on the rigid macroporous polymer. In certain embodiments of the

30      invention, the rigid macroporous polymer having functional moieties capable of

crosslinking to a protein is made by combining a rigid macroporous polymer having reactive epoxide moieties with a nucleophilic compound so that a rigid macroporous polymer having functional moieties capable of crosslinking to a protein is made.

Yet another embodiment of the invention is a method of immobilizing a protein

5      on a rigid macroporous polymer comprising combining a protein having a sulfhydryl, amine, carboxyl or hydroxyl moiety with a rigid macroporous polymer having reactive epoxide moieties under reaction conditions that allow a nucleophilic reaction to occur between the sulfhydryl, amine, carboxyl or hydroxyl moieties on the protein and the epoxide moieties on the rigid macroporous polymer so that the protein is immobilized

10     on the rigid macroporous polymer. In certain embodiments of this method, at least one nucleophilic moiety on the protein is blocked prior to combining the protein with the rigid macroporous polymer.

Analyte sensors of the invention typically incorporate the porous matrices disclosed herein. Typically, the analyte sensor apparatus includes an analyte sensing layer

15     disposed on a conductive layer of the sensor, typically covering a portion or all of the working electrode. This analyte sensing layer detectably alters the electrical current at the working electrode in the conductive layer in the presence of an analyte to be sensed. As disclosed herein, this analyte sensing layer typically includes an enzyme or antibody molecule or the like that reacts with the analyte of interest in a manner that changes the

20     concentrations of a molecule that can modulate the current at the working electrode (see e.g. oxygen and/or hydrogen peroxide as shown in the reaction scheme of FIG. 1). Illustrative analyte sensing layers comprise an enzyme such as glucose oxidase (e.g. for use in glucose sensors) or lactate oxidase (e.g. for use in lactate sensors). In some embodiments of the invention, the analyte sensing layer is disposed upon a porous

25     metallic and/or ceramic and/or polymeric matrix with this combination of elements functioning as an electrode in the sensor.

Typically, the analyte-sensing layer further comprises a carrier protein in a substantially fixed ratio with the analyte sensing compound (e.g. the enzyme) and the analyte sensing compound and the carrier protein are distributed in a substantially

30     uniform manner throughout the analyte sensing layer. Typically the analyte sensing layer

is very thin, for example, less than 1, 0.5, 0.25 or 0.1 microns in thickness. While not
being bound by a specific scientific theory, it is believed that sensors having such thin
analyte sensing layers have surprisingly enhanced characteristics as compared to the
thicker layers that are typically generated by electrodeposition because electrodeposition

5        produces 3-5 micron thick enzyme layers in which only a fraction of the reactive enzyme
within the coating layer is able to access the analyte to be sensed. Such thicker glucose
oxidase pellets that are produced by electrodeposition protocols are further observed to
have a poor mechanical stability (e.g. a tendency to crack) and further take a longer time
to prepare for actual use, typically taking weeks of testing before it is ready for

10       implantation. As these problems are not observed with the thin layered enzyme coatings
described herein, these thin coatings are typical embodiments of the invention.

         In sensors utilizing glucose oxidase for example, the thick coatings produced by
electrodeposition may hinder the ability of hydrogen peroxide generated at the reactive
interface of the 3-5 micron thick enzyme layer to contact the sensor surface and thereby

15       generate a signal. In addition, hydrogen peroxide that is unable to reach a sensor surface
due to such thick coatings can diffuse away from the sensor into the environment in
which the sensor is placed, thereby decreasing the sensitivity and/or biocompatibility of
such sensors. Moreover, while not being bound by a specific scientific theory, it is
believed that sensors having such thin analyte sensing layers have unexpectedly

20       advantageous properties that result from the fact that processes such as spin coating, or
the like, allow for a precise control over the enzyme coating's ratio of glucose oxidase to
albumin (which is used as a carrier protein to stabilize the glucose oxidase in the enzyme
layer). Specifically, because glucose oxidase and albumin have different isoelectric points,
electrodeposition processes may result in a surface coating in which an optimally

25       determined ratio of enzyme to carrier protein is detrimentally altered in the
electrodeposition process, and further wherein the glucose oxidase and the carrier
protein are not distributed in a substantially uniform manner throughout the disposed
enzyme layer. In addition, sensors having such thin analyte sensing layers have
unexpectedly faster response times. While not being bound by a specific scientific

30       theory, it is believed that these surprising and advantageous properties result from the

observation that thin enzyme layers allow better access to the working electrode surface and may allow a greater proportion of the molecules that modulate current at the electrode to access the electrode surface. In this context, in certain sensor embodiments of the invention, an alteration in current in response to exposure to the analyte present in

5      the body of the mammal can be detected via an amperometer within 15, 10, 5 or 2 minutes of the analyte contacting the analyte sensor.

Optionally, the analyte sensing layer has a protein layer disposed thereon and which is typically between this analyte sensing layer and the analyte modulating layer. A protein within the protein layer is an albumin selected from the group consisting of

10     bovine serum albumin and human serum albumin. Typically this protein is crosslinked. Without being bound by a specific scientific theory, it is believed that this separate protein layer enhances sensor function and provides surprising functional benefits by acting as a sort of capacitor that diminishes sensor noise (e.g. spurious background signals). For example, in the sensors of the invention, some amount of moisture may

15     form under the analyte modulating membrane layer of the sensor, the layer which regulates the amount of analyte that can contact the enzyme of the analyte sensing layer. This moisture may create a compressible layer that shifts within the sensor as a patient using the sensor moves. Such shifting of layers within the sensor may alter the way that an analyte such as glucose moves through the analyte sensing layers in a manner that is

20     independent of actual physiological analyte concentrations, thereby generating noise. In this context, the protein layer may act as a capacitor by protecting an enzyme such as GOx from contacting the moisture layer. This protein layer may confer a number of additional advantages such as promoting the adhesion between the analyte sensing layer and the analyte modulating membrane layer. Alternatively, the presence of this layer may

25     result in a greater diffusion path for molecules such as hydrogen peroxide, thereby localizing it to the electrode sensing element and contributing to an enhanced sensor sensitivity.

Typically, the analyte sensing layer and/or the protein layer disposed on the analyte sensing layer has an adhesion promoting layer disposed thereon. Such adhesion

30     promoting layers promote the adhesion between the analyte sensing layer and a proximal

layer, typically an analyte modulating layer. This adhesion promoting layer typically comprises a silane compound such as γ-aminopropyltrimethoxysilane which is selected for its ability to promote optimized adhesion between the various sensor layers and functions to stabilize the sensor. Interestingly, sensors having such a silane containing

5      adhesion promoting layers exhibit unexpected properties including an enhanced overall stability. In addition, silane containing adhesion promoting layers provide a number of advantageous characteristics in addition to an ability to enhancing sensor stability, and can, for example, play a beneficial role in interference rejection as well as in controlling the mass transfer of one or more desired analytes.

10           In certain embodiments of the invention, the adhesion promoting layer further comprises one or more compounds that can also be present in an adjacent layer such as the polydimethyl siloxane (PDMS) compounds that serves to limit the diffusion of analytes such as glucose through the analyte modulating layer. The addition of PDMS to the AP layer for example can be advantageous in contexts where it diminishes the

15      possibility of holes or gaps occurring in the AP layer as the sensor is manufactured.

Typically the adhesion promoting layer has an analyte modulating layer disposed thereon which functions to modulate the diffusion of analytes therethrough. In one embodiment, the analyte modulating layer includes compositions (e.g. polymers and the like) which serve to enhance the diffusion of analytes (e.g. oxygen) through the sensor

20      layers and consequently function to enrich analyte concentrations in the analyte sensing layer. Alternatively, the analyte modulating layer includes compositions which serve to limit the diffusion of analytes (e.g. glucose) through the sensor layers and consequently function to limit analyte concentrations in the analyte sensing layer. An illustrative example of this is a hydrophilic glucose limiting membrane (i.e. functions to limit the

25      diffusion of glucose therethrough) comprising a polymer such as polydimethyl siloxane or the like.

Typically the analyte modulating layer further comprises one or more cover layers which are typically electrically insulating protective layers disposed on at least a portion of the sensor apparatus (e.g. covering the analyte modulating layer). Acceptable polymer

30      coatings for use as the insulating protective cover layer can include, but are not limited

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to, non-toxic biocompatible polymers such as silicone compounds, polyimides, biocompatible solder masks, epoxy acrylate copolymers, or the like. An illustrative cover layer comprises spun on silicone. Typically the cover layer further includes an aperture that exposes at least a portion of a sensor layer (e.g. analyte modulating layer) to a

5      solution comprising the analyte to be sensed.

The analyte sensors described herein can be polarized cathodically to detect, for example, changes in current at the working cathode that result from the changes in oxygen concentration proximal to the working cathode that occur as glucose interacts with glucose oxidase as shown in FIG. 1. Alternatively, the analyte sensors described

10     herein can be polarized anodically to detect for example, changes in current at the working anode that result from the changes in hydrogen peroxide concentration proximal to the working anode that occur as glucose interacts with glucose oxidase as shown in FIG. 1. In typical embodiments of the invention, the current at the working electrode(s) is compared to the current at a reference electrode(s) (a control), with the

15     differences between these measurements providing a value that can then be correlated to the concentration of the analyte being measured. Analyte sensor designs that obtain a current value by obtaining a measurement from a comparison of the currents at these dual electrodes are commonly termed, for example, dual oxygen sensors.

In some embodiments of the invention, the analyte sensor apparatus is designed

20     to function via anodic polarization such that the alteration in current is detected at the anodic working electrode in the conductive layer of the analyte sensor apparatus. Structural design features than can be associated with anodic polarization include designing an appropriate sensor configuration comprising a working electrode which is an anode, a counter electrode which is a cathode and a reference electrode and then

25     selectively disposing the appropriate analyte sensing layer on the appropriate portion of the surface of the anode within this design configuration. Optionally this anodic polarization structural design includes anodes, cathodes and/or working electrodes having different sized surface areas. For example, this structural design includes features where the working electrode (anode) and/or the coated surface of the working electrode

30     is larger than the counter electrode (cathode) and/or the coated surface of the counter

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electrode. In this context, the alteration in current that can be detected at the anodic

working electrode is then correlated with the concentration of the analyte. In certain

illustrative examples of this embodiment of the invention, the working electrode is

measuring and utilizing hydrogen peroxide in the oxidation reaction (see e.g. FIG. 1),

5      hydrogen peroxide that is produced by an enzyme such as glucose oxidase or lactate

oxidase upon reaction with glucose or lactate respectively. Such embodiments of the

invention relating to electrochemical glucose and/or lactate sensors having such

hydrogen peroxide recycling capabilities are particularly interesting because the recycling

of this molecule reduces the amount of hydrogen peroxide that can escape from the

10     sensor into the environment in which it is placed. In this context, implantable sensors

that are designed to reduce the release of tissue irritants such as hydrogen peroxide will

have improved biocompatibility profiles. Moreover as it is observed that hydrogen

peroxide can react with enzymes such as glucose oxidase and compromise their

biological function, such sensors are desired due to their avoidance of this phenomena.

15     Optionally, the analyte modulating layer (e.g. a glucose limiting layer) can include

compositions that serve to inhibit the diffusion of hydrogen peroxide out in to the

environment in which the sensor is placed. Consequently, such embodiments of the

invention improve the biocompatibility of sensors that incorporate enzymes that produce

hydrogen peroxide by incorporating hydrogen peroxide recycling elements disclosed

20     herein.

       Certain embodiments of the analyte sensors of the invention that comprise a base

layer, a conductive layer, an analyte sensing layer, an optional protein layer, an adhesion

promoting layer, an analyte modulating layer and a cover layer exhibit a number of

unexpected properties. For example, in sensors that are structured to function via anodic

25     polarization versus those structured to function via cathodic polarization, differences in

the electrochemical reactions in the analyte sensing layer as well as at the electrode

surface generate and/or consume different chemical entities, thereby altering the

chemical environment in which the various sensor elements function in different

polarities. In this context the sensor structure disclosed herein provides a surprisingly

30     versatile device that is shown to function with an unexpected degree of stability under a

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variety of different chemical and/or electrochemical conditions.

        In certain embodiments of the invention disclosed herein (e.g., those having hydrogen peroxide recycling capabilities) the sensor layer has a plurality of electrodes including a working electrode (e.g. an anode) and a counter electrode (e.g. a cathode),

5     both of which are coated with an analyte sensing layer comprising an enzyme such as glucose oxidase or lactate oxidase. Such sensor designs have surprising properties including an enhanced sensitivity. Without being bound by a specific theory, these properties may result from the enhanced oxidation of hydrogen peroxide at the surface of a working or a counter electrode which produces additional oxygen that can be

10     utilized in the glucose sensing reaction (see, e.g., FIG. 1). Therefore this recycling effect may reduce the oxygen dependent limitations of certain sensor embodiments disclosed herein. Moreover, this design may result in a sensor having a working electrode that can readily reduce available hydrogen peroxide and consequently have a lower electrode potential. Sensors designed to function with lower electrode potentials are typical

15     embodiments of the invention because high electrode potentials in sensors of this type can result in a gas producing hydrolysis reaction which can destabilize the sensors (due to the disruption of sensor layers from gas bubbles produced by hydrolysis reactions). In addition, in sensor embodiments designed so that the counter electrode is coated with a very thin layer of an analyte sensing layer comprising an enzyme such as glucose oxidase

20     or lactate oxidase, the hydrogen peroxide generated in the enzymatic reaction is very close to the reactive surface of the counter electrode. This can increase the overall efficiency of the sensor in a manner that allows for the production of compact sensor designs which include for example, counter electrodes with smaller reactive surfaces.

        A specific illustrative example of an analyte sensor apparatus for implantation

25     within a mammal is a peroxide sensor of the following design. A first layer of the peroxide sensor apparatus is a base layer, typically made from a ceramic such as alumina. A subsequent layer disposed upon the base layer is a conductive layer including a plurality of electrodes including an anodic working electrode and a reference electrode. A subsequent layer disposed on the conductive layer is an analyte sensing layer that

30     includes crosslinked glucose oxidase which senses glucose and consequently generates

hydrogen peroxide as shown in Figure 1.  In the presence of this hydrogen peroxide, the anodic working electrode experiences a measurable increase in current as the hydrogen peroxide generated contacts this anode in the conductive layer and is oxidized.  The reference electrode serves as a control and is physically isolated from the working

5     electrode and the hydrogen peroxide generated according to the reaction shown in Figure 1.  This analyte sensing layer is typically less than 1, 0.5, 0.25 or 0.1 microns in thickness and comprises a mixture of crosslinked human serum albumin in a substantially fixed ratio with the crosslinked glucose oxidase, with the glucose oxidase and the human serum albumin being distributed in a substantially uniform manner throughout the

10    sensor layer.  A subsequent layer disposed on the sensor layer is a protein layer comprising crosslinked human serum albumin.  A subsequent layer disposed on the protein layer is an adhesion promoting layer which promotes the adhesion between the analyte sensing layer and/or the protein layer and an analyte modulating layer which is disposed upon these layers.  This adhesion promoting layer comprises a silane

15    composition.  A subsequent layer disposed on the adhesion promoting layer is the analyte modulating layer in the form of a hydrophilic glucose limiting membrane comprising PDMS which modulates the diffusion of glucose therethrough.  A subsequent layer is a cover layer, typically composed of silicone, which is disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes

20    an aperture that exposes at least a portion of the analyte modulating layer to the external glucose containing environment so that the glucose can access the analyte sensing layer on the working electrode.  This peroxide sensor apparatus functions via anodic polarization such that the hydrogen peroxide signal that is generated by glucose diffusing through the analyte modulating layer and then reacts with the glucose oxidase in the

25    analyte sensing layer creates a detectable change in the current at the anodic working electrode in the conductive layer of the sensor that can be measured by an amperometer. This change in the current at the anodic working electrode can then be correlated with the concentration of glucose in the external environment.  Consequently, a sensor of this design can act as a peroxide based glucose sensor.

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## D.    PERMUTATIONS OF ANALYTE SENSOR APPARATUS AND ELEMENTS

As noted above, the invention disclosed herein includes a number of

5      embodiments including sensors having very thin enzyme coatings.   Such embodiments
of the invention allow artisans to generate a variety of permutations of the analyte sensor
apparatus disclosed herein.   As noted above, illustrative general embodiments of the
sensor disclosed herein include a base layer, a cover layer and at least one layer having a
sensor element such as an electrode disposed between the base and cover layers.

10     Typically, an exposed portion of one or more sensor elements (e.g., a working electrode,
a counter electrode, reference electrode, etc.) is coated with a very thin layer of material
having an appropriate electrode chemistry.   For example, an enzyme such as lactate
oxidase, glucose oxidase, glucose dehydrogenase or hexokinase, can be disposed on the
exposed portion of the sensor element within an opening or aperture defined in the

15     cover layer.   FIG. 2 illustrates a cross-section of a typical sensor structure 100 of the
present invention.   The sensor is formed from a plurality of layers of various conductive
and non-conductive constituents disposed on each other according to a method of the
invention to produce a sensor structure 100.

As noted above, in the sensors of the invention, the various layers (e.g. the

20     analyte sensing layer) of the sensors can have one or more bioactive and/or inert
materials incorporated therein.   The term "incorporated" as used herein is meant to
describe any state or condition by which the material incorporated is held on the outer
surface of or within a solid phase or supporting matrix of the layer.   Thus, the material
"incorporated" may, for example, be immobilized, physically entrapped, attached

25     covalently to functional groups of the matrix layer(s).   Furthermore, any process,
reagents, additives, or molecular linker agents which promote the "incorporation" of said
material may be employed if these additional steps or agents are not detrimental to, but
are consistent with the objectives of the present invention. This definition applies, of
course, to any of the embodiments of the present invention in which a bioactive

30     molecule (e.g. an enzyme such as glucose oxidase) is "incorporated." For example,
certain layers of the sensors disclosed herein include a proteinaceous substance such as

albumin which serves as a crosslinkable matrix. As used herein, a proteinaceous
substance is meant to encompass substances which are generally derived from proteins
whether the actual substance is a native protein, an inactivated protein, a denatured
protein, a hydrolyzed species, or a derivatized product thereof. Examples of suitable
5    proteinaceous materials include, but are not limited to enzymes such as glucose oxidase
and lactate oxidase and the like, albumins (e.g. human serum albumin, bovine serum
albumin etc.), caseins, gamma-globulins, collagens and collagen derived products (e.g.,
fish gelatin, fish glue, animal gelatin, and animal glue).

An illustrative embodiment of the invention is shown in FIG. 2. This
10   embodiment includes an electrically insulating base layer 102 to support the sensor 100.
The electrically insulating layer base 102 can be made of a material such as a ceramic
substrate, which may be self-supporting or further supported by another material as is
known in the art. In an alternative embodiment, the electrically insulating layer 102
comprises a polyimide substrate, for example a polyimide tape, dispensed from a reel.
15   Providing the layer 102 in this form can facilitate clean, high density mass production.
Further, in some production processes using such a polyimide tape, sensors 100 can be
produced on both sides of the tape.

Typical embodiments of the invention include an analyte sensing layer disposed
on the base layer 102. In an illustrative embodiment as shown in FIG. 2 the analyte
20   sensing layer comprises a conductive layer 104 which is disposed on insulating base layer
102. Typically the conductive layer 104 comprises one or more electrodes. The
conductive layer 104 can be applied using many known techniques and materials as will
be described hereafter, however, the electrical circuit of the sensor 100 is typically
defined by etching the disposed conductive layer 104 into a desired pattern of conductive
25   paths. A typical electrical circuit for the sensor 100 comprises two or more adjacent
conductive paths with regions at a proximal end to form contact pads and regions at a
distal end to form sensor electrodes. An electrically insulating protective cover layer 106
such as a polymer coating is typically disposed on portions of the conductive layer 104.
Acceptable polymer coatings for use as the insulating protective layer 106 can include,
30   but are not limited to, non-toxic biocompatible polymers such as polyimide,

biocompatible solder masks, epoxy acrylate copolymers, or the like. Further, these coatings can be photo-imageable to facilitate photolithographic forming of apertures 108 through to the conductive layer 104. In certain embodiments of the invention, an analyte sensing layer is disposed upon a porous metallic and/or ceramic and/or

5   polymeric matrix with this combination of elements functioning as an electrode in the sensor.

In the sensors of the present invention, one or more exposed regions or apertures 108 can be made through the protective layer 106 to the conductive layer 104 to define the contact pads and electrodes of the sensor 100. In addition to

10  photolithographic development, the apertures 108 can be formed by a number of techniques, including laser ablation, chemical milling or etching or the like. A secondary photoresist can also be applied to the cover layer 106 to define the regions of the protective layer to be removed to form the apertures 108. An operating sensor 100 typically includes a plurality of electrodes such as a working electrode and a counter

15  electrode electrically isolated from each other, however typically situated in close proximity to one another. Other embodiments may also include a reference electrode. Still other embodiments may utilize a separate reference element not formed on the sensor. The exposed electrodes and/or contact pads can also undergo secondary processing through the apertures 108, such as additional plating processing, to prepare

20  the surfaces and/or strengthen the conductive regions.

An analyte sensing layer 110 is typically disposed on one or more of the exposed electrodes of the conductive layer 104 through the apertures 108. Typically, the analyte sensing layer 110 is a sensor chemistry layer and most typically an enzyme layer. Typically, the analyte sensing layer 110 comprises the enzyme glucose oxidase or the

25  enzyme lactate oxidase. In such embodiments, the analyte sensing layer 110 reacts with glucose to produce hydrogen peroxide which modulates a current to the electrode which can be monitored to measure an amount of glucose present. The sensor chemistry layer 110 can be applied over portions of the conductive layer or over the entire region of the conductive layer. Typically the sensor chemistry layer 110 is disposed on portions of a

30  working electrode and a counter electrode that comprise a conductive layer. Some

methods for generating the thin sensor chemistry layer 110 include spin coating

processes, dip and dry processes, low shear spraying processes, ink-jet printing processes,

silk screen processes and the like. Most typically the thin sensor chemistry layer 110 is

applied using a spin coating process.

5          The analyte sensing layer 110 is typically coated with one or more coating layers.

In some embodiments of the invention, one such coating layer includes a membrane

which can regulate the amount of analyte that can contact an enzyme of the analyte

sensing layer. For example, a coating layer can comprise an analyte modulating

membrane layer such as a glucose limiting membrane which regulates the amount of

10         glucose that contacts the glucose oxidase enzyme layer on an electrode. Such glucose

limiting membranes can be made from a wide variety of materials known to be suitable

for such purposes, e.g., silicone, polyurethane, polyurea cellulose acetate, Nafion,

polyester sulfonic acid (Kodak AQ), hydrogels or any other membrane known to those

skilled in the art.

15         In some embodiments of the invention, a coating layer is a glucose limiting

membrane layer 112 which is disposed above the sensor chemistry layer 110 to regulate

glucose contact with the sensor chemistry layer 110. In some embodiments of the

invention, an adhesion promoter layer 114 is disposed between the membrane layer 112

and the sensor chemistry layer 110 as shown in FIG. 2 in order to facilitate their contact

20         and/or adhesion. The adhesion promoter layer 114 can be made from any one of a wide

variety of materials known in the art to facilitate the bonding between such layers.

Typically, the adhesion promoter layer 114 comprises a silane compound. In alternative

embodiments, protein or like molecules in the sensor chemistry layer 110 can be

sufficiently crosslinked or otherwise prepared to allow the membrane layer 112 to be

25         disposed in direct contact with the sensor chemistry layer 110 in the absence of an

adhesion promoter layer 114.

As noted above, embodiments of the present invention can include one or more

functional coating layers. As used herein, the term "functional coating layer" denotes a

layer that coats at least a portion of at least one surface of a sensor, more typically

30         substantially all of a surface of the sensor, and that is capable of interacting with one or

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more analytes, such as chemical compounds, cells and fragments thereof, etc., in the
environment in which the sensor is disposed. Non-limiting examples of functional
coating layers include sensor chemistry layers (e.g., enzyme layers), analyte limiting layers,
biocompatible layers; layers that increase the slipperiness of the sensor; layers that
5   promote cellular attachment to the sensor; layers that reduce cellular attachment to the
sensor; and the like. Typically analyte modulating layers operate to prevent or restrict the
diffusion of one or more analytes, such as glucose, through the layers. Optionally such
layers can be formed to prevent or restrict the diffusion of one type of molecule through
the layer (e.g. glucose), while at the same time allowing or even facilitating the diffusion
10  of other types of molecules through the layer (e.g. $O_2$). An illustrative functional coating
layer is a hydrogel such as those disclosed in U.S. Patent Nos. 5,786,439 and 5,391,250,
the disclosures of each being incorporated herein by reference. The hydrogels described
therein are particularly useful with a variety of implantable devices for which it is
advantageous to provide a surrounding water layer.
15      The sensor embodiments disclosed herein can include layers having UV-
absorbing polymers. In accordance with one aspect of the present invention, there is
provided a sensor including at least one functional coating layer including an UV-
absorbing polymer. In some embodiments, the UV-absorbing polymer is a polyurethane,
a polyurea or a polyurethane/polyurea copolymer. More typically, the selected UV-
20  absorbing polymer is formed from a reaction mixture including a diisocyanate, at least
one diol, diamine or mixture thereof, and a polyfunctional UV-absorbing monomer.
        UV-absorbing polymers are used with advantage in a variety of sensor fabrication
methods, such as those described in U.S. Pat. No. 5,390,671, to Lord et al., entitled
"Transcutaneous Sensor Insertion Set"; No. 5,165,407, to Wilson et al., entitled
25  "Implantable Glucose Sensor"; and U.S. Pat. No. 4,890,620, to Gough, entitled "Two-
Dimensional Diffusion Glucose Substrate Sensing Electrode", which are incorporated
herein in their entireties by reference. However, any sensor production method which
includes the step of forming an UV-absorbing polymer layer above or below a sensor
element is considered to be within the scope of the present invention. In particular, the
30  inventive methods are not limited to thin-film fabrication methods, and can work with

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other sensor fabrication methods that utilize UV-laser cutting. Embodiments can work with thick-film, planar or cylindrical sensors and the like, and other sensor shapes requiring laser cutting.

5          As disclosed herein, the sensors of the present invention are particularly designed for use as subcutaneous or transcutaneous glucose sensors for monitoring blood glucose levels in a diabetic patient. Typically each sensor comprises a plurality of sensor elements, for example electrically conductive elements such as elongated thin film conductors, formed between an underlying insulative thin film base layer and an overlying insulative thin film cover layer.

10          If desired, a plurality of different sensor elements can be included in a single sensor. For example, both conductive and reactive sensor elements can be combined in one sensor, optionally with each sensor element being disposed on a different portion of the base layer. One or more control elements can also be provided. In such embodiments, the sensor can have defined in its cover layer a plurality of openings or

15     apertures. One or more openings can also be defined in the cover layer directly over a portion of the base layer, in order to provide for interaction of the base layer with one or more analytes in the environment in which the sensor is disposed. The base and cover layers can be comprised of a variety of materials, typically polymers. In more specific embodiments the base and cover layers are comprised of an insulative material such as a

20     polyimide. Openings are typically formed in the cover layer to expose distal end electrodes and proximal end contact pads. In a glucose monitoring application, for example, the sensor can be placed transcutaneously so that the distal end electrodes are in contact with patient blood or extracellular fluid, and the contact pads are disposed externally for convenient connection to a monitoring device.

25          The sensors of the invention can have any desired configuration, for example planar or cylindrical. The base layer 102 can be self-supportive, such as a rigid polymeric layer, or non-self supportive, such as a flexible film. The latter embodiment is desirable in that it permits continuous manufacture of sensors using, for example, a roll of a polymeric film which is continuously unwound and upon which sensor elements and

30     coating layers are continuously applied.

A general embodiment of the invention is a sensor designed for implantation within a body that comprises a base layer, an analyte sensing layer disposed upon the base layer which includes a plurality of sensor elements, an enzyme layer (typically less than 2 microns in thickness) disposed upon the analyte sensing layer which coats all of

5      the plurality of sensing elements on the conductive layer, and one or more coating layers. Typically the enzyme layer comprises glucose oxidase; typically in a substantially fixed ratio with a carrier protein. In a specific embodiment, the glucose oxidase and the carrier protein are distributed in a substantially uniform manner throughout the disposed enzyme layer. Typically the carrier protein comprises albumin, typically in an amount of

10     about 5% by weight. As used herein, "albumin" refers to those albumin proteins typically used by artisans to stabilize polypeptide compositions such as human serum albumin, bovine serum albumin and the like. In some embodiments of the invention, a coating layer is an analyte contacting layer which is disposed on the sensor so as to regulate the amount of analyte that can contact the enzyme layer. In further

15     embodiments, the sensor includes an adhesion promoter layer disposed between the enzyme layer and the analyte contacting layer; and, the enzyme layer is less than 1, 0.5, 0.25 or 0.1 microns in thickness.

One aspect of the present invention involves processes for making sensors having improved electrode chemistry coatings (e.g., enzyme coatings of less than 2

20     microns in thickness) with enhanced material properties. Methods for producing the extremely thin enzyme coatings of the invention include spin coating processes, dip and dry processes, low shear spraying processes, ink-jet printing processes, silk screen processes and the like. Typically, such coatings are vapor crosslinked subsequent to their application. Surprisingly, sensors produced by these processes have material properties

25     that exceed those of sensors having coatings produced by electrodeposition including enhanced longevity, linearity, regularity as well as improved signal to noise ratios. In addition, certain sensor embodiments of the invention that utilize glucose oxidase ·coatings formed by such processes are designed to recycle hydrogen peroxide and improve the biocompatibility profiles of such sensors. Illustrative embodiments of the

30     invention include those designed to both consume hydrogen peroxide and recycle

oxygen.

In this context, an illustrative embodiment of the invention is a method of making a less than about 2 micron coating of stabilized glucose oxidase on the surface of a matrix such as an electrode comprising combining glucose oxidase with albumin in a

5    fixed ratio (one that is typically optimized for glucose oxidase stabilizing properties) and applying the glucose oxidase and albumin mixture to the surface of the matrix by a process selected from the group consisting of a spin coating process, a dip and dry process, a microdeposition process, a jet printer deposition process, a screen printing process or a doctor blading process. Typically the stabilized glucose oxidase coating is

10   applied to the surface of an electrode by a spin coating process. In some embodiments, the glucose oxidase/albumin is prepared in a physiological solution (e.g., phosphate buffered saline at neutral pH) with the albumin being present in an amount of about 5% albumin by weight. Optionally the stabilized glucose oxidase layer that is formed on the conductive layer is less than 2, 1, 0.5, 0.25 or 0.1 microns in thickness. A closely related

15   embodiment of the invention is a stabilized glucose oxidase layer for coating the surface of an electrode wherein the glucose oxidase is mixed with a carrier protein in a fixed ratio within the layer, the glucose oxidase and the carrier protein are distributed in a substantially uniform manner throughout the layer. Typically the layer is less than 2 microns in thickness.

20   Embodiments of the invention include a design where an analyte sensing layer is disposed upon a porous metallic and/or ceramic and/or polymeric matrix with this combination of elements functioning as an electrode in the sensor. A related embodiment of the invention is an electrochemical analyte sensor which includes a base layer, a conductive layer disposed upon the base layer that includes at least one working

25   electrode and at least one counter electrode, an analyte sensing layer disposed upon the conductive layer, wherein the analyte sensing layer is less than 2 microns in thickness; and an analyte modulating layer that regulates the amount of analyte that contacts the enzyme layer, typically by limiting the amount of analyte that can diffuse through the layer and contact the analyte sensing layer. In an optional embodiment of the invention,

30   the working electrode and/or the coated surface of the working electrode is larger than

counter electrode and/or the coated surface of the counter electrode. In some

embodiments, the enzyme layer comprises glucose oxidase stabilized by coating it on the

working electrode and the counter electrode in combination with a carrier protein in a

fixed ratio. In one embodiment, this glucose oxidase enzyme layer substantially covers

5       the conductive layer. Embodiments where the glucose oxidase enzyme layer is disposed

in a uniform coating over the whole conductive layer are typical because they may avoid

problems associated with sensors having multiple different coatings on a single layer such

as the selective delamination of different coatings having different material properties.

Typically, the sensor includes an adhesion promoting layer disposed between the enzyme

10      layer and the analyte modulating layer.

A related embodiment of the invention is an electrochemical analyte sensor

which includes a base layer, a conductive layer disposed upon the base layer that includes

at least one working electrode, at least one reference electrode and at least one counter

electrode, an enzyme layer disposed upon the conductive layer, and an analyte

15      modulating cover layer that regulates the amount of analyte that contacts the enzyme

layer. In some embodiments, the enzyme layer is less than 2 microns in thickness and is

coated on at least a portion of the working electrode, the reference electrode and the

counter electrode. In an illustrative embodiment, the enzyme layer substantially covers

the working electrode, the reference electrode and the counter electrode. Optionally, the

20      enzyme layer comprises glucose oxidase in combination with a carrier protein (e.g.

albumin) in a fixed ratio. Typically, the sensor includes an adhesion promoting layer

disposed between the enzyme layer and the analyte modulating layer.

Yet another embodiment of the invention comprises a glucose sensor for

implantation within a body which includes a base layer, a conductive layer disposed upon

25      the base layer, an analyte sensing layer comprising glucose oxidase disposed upon the

conductive layer, wherein the glucose oxidase is stabilized by combining it with albumin

in a defined ratio and further wherein the glucose oxidase and the albumin are distributed

in a substantially uniform manner throughout the disposed layer, and a glucose limiting

layer that regulates the amount of glucose that diffuses through the glucose limiting layer

30      and contacts the glucose oxidase layer. In some embodiments, the conductive layer

includes a plurality of sensor elements including at least one working electrode and at least one counter electrode. In such sensor embodiments, the analyte sensing layer comprising glucose oxidase is typically less than 2, 1, 0.5, 0.25 or 0.1 microns in thickness and the albumin in the layer is present in an amount of about 5% albumin by weight.

5    Typically the sensor includes an adhesion promoting layer disposed between the analyte sensing layer comprising glucose oxidase and the glucose limiting layer.

## E.    ANALYTE SENSOR APPARATUS CONFIGURATIONS

10    In a clinical setting, accurate and relatively fast determinations of analytes such as glucose and/or lactate levels can be determined from blood samples utilizing electrochemical sensors. Conventional sensors are fabricated to be large, comprising many serviceable parts, or small, planar-type sensors which may be more convenient in many circumstances. The term "planar" as used herein refers to the well-known

15    procedure of fabricating a substantially planar structure comprising layers of relatively thin materials, for example, using the well-known thick or thin-film techniques. See, for example, Liu et al., U.S. Pat. No. 4,571,292, and Papadakis et al., U.S. Pat. No. 4,536,274, both of which are incorporated herein by reference. As noted below, embodiments of the invention disclosed herein have a wider range of geometrical configurations (e.g.

20    planar) than existing sensors in the art. In addition, certain embodiments of the invention include one or more of the sensors disclosed herein coupled to another apparatus such as a medication infusion pump.

Figure 2 provides a diagrammatic view of a typical analyte sensor configuration of the current invention. Certain sensor configurations are of a relatively flat "ribbon"

25    type configuration that can be made with the analyte sensor apparatus. Such "ribbon" type configurations illustrate an advantage of the sensors disclosed herein that arises due to the spin coating of sensing enzymes such as glucose oxidase, a manufacturing step that produces extremely thin enzyme coatings that allow for the design and production of highly flexible sensor geometries. Such thin enzyme coated sensors provide further

30    advantages such as allowing for a smaller sensor area while maintaining sensor sensitivity, a highly desirable feature for implantable devices (e.g. smaller devices are easier to

implant). Consequently, sensor embodiments of the invention that utilize very thin analyte sensing layers that can be formed by processes such as spin coating can have a wider range of geometrical configurations (e.g. planar) than those sensors that utilize enzyme layers formed via processes such as electrodeposition.

5        Certain sensor configurations include multiple conductive elements such as multiple working, counter and reference electrodes. Advantages of such configurations include increased surface area which provides for greater sensor sensitivity. For example, one sensor configuration introduces a third working sensor. One obvious advantage of such a configuration is signal averaging of three sensors which increases 10    sensor accuracy. Other advantages include the ability to measure multiple analytes. In particular, analyte sensor configurations that include electrodes in this arrangement (e.g. multiple working, counter and reference electrodes) can be incorporated into multiple analyte sensors. The measurement of multiple analytes such as oxygen, hydrogen peroxide, glucose, lactate, potassium, calcium, and any other physiologically relevant 15    substance/analyte provides a number of advantages, for example the ability of such sensors to provide a linear response as well as ease in calibration and/or recalibration.

An exemplary multiple sensor device comprises a single device having a first sensor which is polarized cathodically and designed to measure the changes in oxygen concentration that occur at the working electrode (a cathode) as a result of glucose 20    interacting with glucose oxidase; and a second sensor which is polarized anodically and designed to measure changes in hydrogen peroxide concentration that occurs at the working electrode (an anode) as a result of glucose coming form the external environment and interacting with glucose oxidase. As is known in the art, in such designs, the first oxygen sensor will typically experience a decrease in current at the 25    working electrode as oxygen contacts the sensor while the second hydrogen peroxide sensor will typically experience an increase in current at the working electrode as the hydrogen peroxide generated as shown in Figure 1 contacts the sensor. In addition, as is known in the art, an observation of the change in current that occurs at the working electrodes as compared to the reference electrodes in the respective sensor systems 30    correlates to the change in concentration of the oxygen and hydrogen peroxide

molecules which can then be correlated to the concentration of the glucose in the external environment (e.g. the body of the mammal).

The analyte sensors of the invention can be coupled with other medical devices such as medication infusion pumps. In a illustrative variation of this scheme, replaceable

5   analyte sensors of the invention can be coupled with other medical devices such as medication infusion pumps, for example by the use of a port couple to the medical device (e.g. a subcutaneous port with a locking electrical connection).

**II.    ILLUSTRATIVE METHODS AND MATERIALS FOR MAKING**
10          **ANALYTE SENSOR APPARATUS OF THE INVENTION**

A number of articles, U.S. patents and patent application describe the state of the art with the common methods and materials disclosed herein and further describe various elements (and methods for their manufacture) that can be used in the sensor

15   designs disclosed herein. These include for example, U.S. Patent Nos. 6,413,393; 6,368,274; 5,786,439; 5,777,060; 5,391,250; 5,390,671; 5,165,407, 4,890,620, 5,390,671, 5,390,691, 5,391,250, 5,482,473, 5,299,571, 5,568,806; United States Patent Application 20020090738; as well as PCT International Publication Numbers WO 01/58348, WO 03/034902, WO 03/035117, WO 03/035891, WO 03/023388, WO 03/022128, WO

20   03/022352, WO 03/023708, WO 03/036255, WO03/036310 and WO 03/074107, the contents of each of which are incorporated herein by reference.

Typical sensors for monitoring glucose concentration of diabetics are further described in Shichiri, et al.,: "In Vivo Characteristics of Needle-Type Glucose Sensor-Measurements of Subcutaneous Glucose Concentrations in Human Volunteers," Horm.

25   Metab. Res., Suppl. Ser. 20:17-20 (1988); Bruckel, et al.,: "In Vivo Measurement of Subcutaneous Glucose Concentrations with an Enzymatic Glucose Sensor and a Wick Method," Klin. Wochenschr. 67:491-495 (1989); and Pickup, et al.,: "In Vivo Molecular Sensing in Diabetes Mellitus: An Implantable Glucose Sensor with Direct Electron Transfer," Diabetologia 32:213-217 (1989). Other sensors are described in, for example

30   Reach, et al., in ADVANCES IN IMPLANTABLE DEVICES, A. Turner (ed.), JAI Press, London, Chap. 1, (1993), incorporated herein by reference.

A.      GENERAL METHODS FOR MAKING ANALYTE SENSORS

A typical embodiment of the invention disclosed herein is a method of making a
sensor apparatus for implantation within a mammal comprising the steps of: providing a
5       base layer; forming a conductive layer on the base layer, wherein the conductive layer
includes an electrode (and typically a working electrode, a reference electrode and a
counter electrode); forming an analyte sensing layer on the conductive layer, wherein the
analyte sensing layer includes a composition that can alter the electrical current at the
electrode in the conductive layer in the presence of an analyte; optionally forming a
10      protein layer on the analyte sensing layer; forming an adhesion promoting layer on the
analyte sensing layer or the optional protein layer; forming an analyte modulating layer
disposed on the adhesion promoting layer, wherein the analyte modulating layer includes
a composition that modulates the diffusion of the analyte therethrough; and forming a
cover layer disposed on at least a portion of the analyte modulating layer, wherein the
15      cover layer further includes an aperture over at least a portion of the analyte modulating
layer. In certain embodiments of these methods, the analyte sensor apparatus is formed
in a planar geometric configuration

As disclosed herein, the various layers of the sensor can be manufactured to
exhibit a variety of different characteristics which can be manipulated according to the
20      specific design of the sensor. For example, the adhesion promoting layer includes a
compound selected for its ability to stabilize the overall sensor structure, typically a silane
composition. In some embodiments of the invention, the analyte sensing layer is formed
by a spin coating process and is of a thickness selected from the group consisting of less
than 1, 0.5, 0.25 and 0.1 microns in height.

25      Typically, a method of making the sensor includes the step of forming a protein
layer on the analyte sensing layer, wherein a protein within the protein layer is an albumin
selected from the group consisting of bovine serum albumin and human serum albumin.
Typically, a method of making the sensor includes the step of forming an analyte sensing
layer that comprises an enzyme composition selected from the group consisting of
30      glucose oxidase, glucose dehydrogenase, lactate oxidase, hexokinase and lactate

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dehydrogenase. In such methods, the analyte sensing layer typically comprises a carrier protein composition in a substantially fixed ratio with the enzyme and the enzyme and the carrier protein are distributed in a substantially uniform manner throughout the analyte sensing layer.

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B.      TYPICAL METHODS FOR MAKING POROUS ENZYME MATRICES

One embodiment of the invention comprises porous metallic matrices. Typically, metallic substrate embodiments of the invention can be manufactured with the desired porosity, pore-size distribution, and tortuosity through a printing process. The

10      metallic substrate may either be printed as a film or within the confines of a mold, either directly in place onto the sensor assembly or onto a temporary substrate. The ink can consist of fine metallic particles suspended in a porogenic carrier. The metallic particles may consist of a single pure metal or alloy. Different types of metallic particles may also be printed either at the same time to form a mixture, or at different times to form layers.

15      The porogenic carrier can consist of a solvent with or without various polymers, glasses, ceramics, and/or frit materials. The mold may consist of various ceramics, polymers, or metals. Many thin layers of ink may need to be printed in order to fill the mold or to obtain a film of the desired thickness. To remove the solvents, the printed metallic matrix can be dried at an appropriate temperature. The resulting porous bed of metallic

20      powder can then be fired approximately in the range of 350°C-2,000°C to bond the metallic and, if any, ceramic particles together. This can form a highly porous and tortuous metallic substrate onto which an enzyme such as GOx can be immobilized. If desired, the morphology of the metallic substrate can be adjusted by manipulating the size of the metallic particles as well as the composition of the porogenic carrier.

25      Additionally, various glass, ceramic, and/or metallic particles included in the ink can be etched from the printed material to create pores using materials such as, but not limited to, hydrofluoric acid and sodium hydroxide. Prior to coating the metallic substrate with glucose oxidase, platinum black may or may not be plated using standard techniques.

The past few years have seen increasing interest in porous metallic materials,

30      especially in foams made of metals such as aluminum or aluminum alloys. Consequently,

in certain embodiments of the invention, the matrix may comprise a metallic foam. Porous metals are those that contain a multitude of pores, i.e. closed, curved gas voids with a smooth surface. Metal(lic) foams are special cases of porous metals. A solid foam originates from a liquid foam in which gas bubbles are finely dispersed in a liquid. In a

5      metal sponge, space is filled by pieces of metal that form a continuous network and co-exist with a network of empty space which is also interconnected. Illustrative materials of this type are described for example in: Cellular Metals: Manufacture, Properties and Applications: J. Banhart, N.A. Fleck, A. Mortensen (Editors); and Proceedings of the 3rd International Conference on Cellular Metals and Metal Foaming Technology (MetFoam

10     2003), J. Banhart, M.F. Ashby, N.A. Fleck (Editors), the contents of which are incorporated herein by reference.

        In an alternate embodiment, the porous metallic substrates can be manufactured by drilling small holes into a metal sheet, film, foil, rod, or block using a laser beam or some other type of drilling technology. In another embodiment, a woven wire mesh can

15     be used as a porous metallic substrate. For example, the fabrication of 3-D micromesh Ni Structures using electroplating has been described in the art such as fabrication methods of a 3-D micromesh Ni electrode. Specifically, inverse-micromesh photoresist structures, fabricated by multiple inclined backside exposure, can be used as a mold for Ni electroplating, with Ni meshes of about 3μm in diameter obtained by this method.

20     The enzyme composition can be applied to the porous matrices by any one of a variety of methods known in the art. In one illustrative embodiment, an enzyme such as glucose oxidase can be dissolved in a solvent and dip, spray, or spin coated onto the porous metallic substrate. For some substrate geometries and morphologies, it may be desirable to instead pump the enzyme solution through the pores. The coating solvent

25     may consist of aqueous buffer and/or various organic solvents and/or surfactants including, but not limited to, various alcohols, dimethyl sulfoxide, and polyoxyethylene(20)sorbitan monolaurate ("Tween™ 20"). Ingress of the protein into porous substrates may be promoted by decreasing the viscosity of the enzyme solution through the manipulation of its composition and/or by applying vacuum and/or

30     centrifugation and/or ultrasonic vibration to the coated substrate. Other bio and/or

synthetic polymers may also be coated along with the enzyme as filler material such as,
but not limited to: bovine serum albumin, human serum albumin, polyethylene glycol,
and O',O'-Bis(2-aminopropyl)polyethylene glycol ("Jeffamine®"). The coated enzyme
and filler materials (if any) will be immobilized onto the metallic substrate using an

5      appropriate homobifunctional (i.e. glutaraldehyde or disuccinimidyl suberate),
heterobifunctional (i.e. succinimidyl-4-[N-maleimidomethyl] cyclohexane-1-carboxylate),
trifunctional (i.e. 4-azido-2-nitrophenylbiocytin-4-nitrophenyl ester), and/or zero-length
(i.e. 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) cross-linking agent
or agents that could be selected by individuals well versed in fields of protein

10     immobilization, bioconjugate techniques, or polymer chemistry.

       In an alternate embodiment, a process provided by SurModics Inc. under the
trademark *PHOTOLINK*$^{TM}$ can be used to immobilize an enzyme such as glucose oxidase
onto the porous metallic substrate. Such *PHOTOLINK*$^{TM}$ methods are set forth in U.S.
Pat. Nos. 3,959,078, 4,722,906, 5,229,172; 5,308,641; 5,350,800 and 5,415,938.

15     As disclosed herein, other embodiments of the invention include an essentially
rigid, non-swelling porous enzyme-polymer matrix. In this context, molded continuous
rods of macroporous polymers have been developed for use as chromatographic
separation media (see, e.g. U.S. Patent No. 5,453,185 and PCT Publication No. WO
93/07945, the contents of which are incorporated herein by reference). Examples

20     include, but are not limited to poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) and
poly(styrene-*co*-divinylbenzene). As disclosed in U.S. Patent No. 5,453,185, a typical
polymerization mixture at a minimum contains at least one polyvinyl monomer, a free
radical generating initiator, and a porogen. The mixture may also contain one or more
monovinyl monomers and/or soluble polymers or insoluble macroporous polymer

25     particles. Suitable polyvinyl monomers include divinylbenzene, divinylnaphthalene,
divinylpyridine, alkylene dimethacrylates, hydroxyalkylene dimethacrylates,
hydroxyalkylene diacrylates, oligoethylene glycol dimethacrylates, oligoethylene glycol
diacrylates, vinyl esters of polycarboxylic acids, divinyl ether, pentaerythritol di-, tri-, or
tetramethacrylate or acrylate, trimethylopropane trimethacrylate or acrylate, alkylene bis

30     acrylamides or methacrylamides, and mixtures of any such suitable polyvinyl monomers.

The alkylene groups generally contain about 1-6 carbon atoms. Monovinyl monomers which may be used include styrene, ring substituted styrenes wherein the substituents include chloromethyl, alkyl with up to 18 carbon atoms, hydroxyl, t-butyloxycarbonyl, halogen, nitro, amino group, protected hydroxyls or amino groups, vinylnaphthalene,

5    acrylates, methacrylates, vinylacetate, vinylpyrolidone, and mixtures thereof. The polyvinyl monomer or polyvinyl monomer plus the monovinyl monomer are generally present in the polymerization mixture in an amount of from about 10 to 60 vol. %, and more typically in an amount of from about 20 to 40 vol. %. The porogen that is used may be selected from a variety of different types of materials. For example, suitable liquid

10   porogens include aliphatic hydrocarbons, aromatic hydrocarbons, esters, alcohols, ketones, ethers, solutions of soluble polymers, and mixtures thereof. The porogen is generally present in the polymerization mixture in an amount of from about 40 to 90 vol %, more typically from about 60 to 80 vol %. Soluble polymers and insoluble polymer particles may be employed in combination with the monomers. These polymers are

15   added to the polymerization mixture prior to polymerization. The soluble polymers are dissolved out of the plug after its formation by passing a solvent through the plug. The soluble polymers serve as a polymeric porogen to increase the porosity of the final plug. Suitable soluble polymers used herein include non-crosslinked polymers or copolymers of such monomers as styrene or ring substituted styrene, acrylates, methacrylates, dienes,

20   vinylchloride, and vinylacetate. The insoluble polymer particles are used to reduce the volume shrinkage during the polymerization. The lesser the volume of the monomers in the polymerization mixture the smaller the contraction of volume upon polymerization. Suitable insoluble polymer particles used herein include macroporous polymer particles which are cross-linked copolymers of the same monomers. It is, however, common due

25   to compatibility to employ insoluble polymer particles which are formed from the same monomers used to form the polymerization mixture with which they are to be combined. The polymer particles initially have a diameter of from about 1 to 1,000 micrometers. It is not necessary that the mixture of polymer particles have the same particle size. In fact, it is more economical, and, therefore common to use irregularly sized polymer particles.

30   While not necessary, the polymer particles may be soaked with a liquid immiscible with

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the polymerization mixture which can contain an inhibitor which inhibits free radical
polymerization. This is done in order to prevent polymerization in the inside of the
macroporous particles which would cause filling of the pores and would effectively
remove them from the separation process. The rod would then contain nonporous pools

5   unable to contribute to the separation process. Suitable inhibitors include cupric chloride
and sodium nitrite. The inhibitor is generally present in an amount of from about 0,001
to 1 wt %, and more typically in an amount of from about 0.1 to 1 wt %, based on the
total weight of particles. The polymer particles are typically degassed prior to use in the
polymerization mixture. This may be accomplished by any of the conventional means

10  known in the art. It, however, is typical to soak the particles in water, optionally
containing a polymerization inhibitor, and remove the air from the pores by keeping the
water-polymer particle mixture under the vacuum of a water pump for a suitable period
of time such as about 5 to 20 minutes. Excess water may then be removed by filtering.
The soluble polymers are generally present in an amount of from about 5 to 40% by

15  volume of the polymerization mixture and the insoluble polymer particles in an amount
of from about 5 to 50% by volume. Conventional free-radical generating polymerization
initiators may be employed to initiate polymerization. Examples of suitable initiators
include peroxides such as OO-t-amyl-O-(2-ethylhexyl)monoperoxycarbonate,
dipropylperoxydicarbonate, and benzoyl peroxide, as well as azo compounds such as

20  azobisisobutyronitrile, 2,2'-azobis(2-amidinopropane)dihydrochloride, and 2,2'-
azobis(isobutyramide)dihydrate. It has been found that the choice of initiator may be
used as a means to control the pore distribution in a plug. The initiator is generally
present in the polymerization mixture in an amount of from about 0.2 to 5% by weight
of the monomers.

25          Polymers useful for making the essentially rigid, non-swelling porous enzyme-
polymer matrices are essentially incompressible and do not change their overall size in
response to changes in their solvating environment. Adjustments to the polymerization
conditions can be used to control the morphology of the pores. Hence, highly porous
(50-70%) polymers can be created that possess significant volume fractions of pores in

30  the ranges of 1-100 nm and 100-3,000 nm (i.e. 20% and 80%, respectively). Polymers

with this type of pore structure possess a very high specific surface area (i.e. 185 m²/g), and are expected to allow for high enzyme immobilization densities (1-100 mg/mL).

In an illustrative embodiment of the rigid, non-swelling porous enzyme-polymer matrices, a nucleophilic compound can be used to functionalize a macroporous, rigid polymer that possesses reactive epoxide groups. A cross-linking agent can then be used to immobilize the bio-sensing enzyme to the polymer via the functional groups of the enzyme and polymer substrate. Other nucleophilic compounds that can be used to functionalize epoxide-activated polymers include, but are not limited to ammonia, ethylenediamine, ethanolamine, carbohydrates, cysteine, and other amino acids. For a given enzyme and functionalized polymer combination, an appropriate homobifunctional (i.e. disuccinimidyl suberate), heterobifunctional (i.e. succinimidyl-4-[N-maleimidomethyl] cyclohexane-1-carboxylate), trifunctional (i.e. 4-azido-2-nitrophenylbiocytin-4-nitrophenyl ester), and/or zero-length (i.e. 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) cross-linking agent or agents could be selected by individuals well versed in fields of protein immobilization or bioconjugate techniques.

In another embodiment of the rigid, non-swelling porous enzyme-polymer matrices, the bio-sensing enzyme will be directly immobilized onto an epoxide-activated polymer via nucleophilic attack by sulfhydryl, amine, hydroxyl, and/or carboxyl groups that are either native to the enzyme, or have been added to the wild-type peptide sequence via genetic engineering or directed evolution. If desired, the nucleophilic functional groups of the enzyme may be reversibly or irreversibly blocked or protected during the immobilization, using compounds that would be familiar to anyone well versed in protein conjugation (i.e. 5,5'-dithio-bis-[2-nitrobenzoic acid] or N-ethylmaleimide).

In another embodiment of the rigid, non-swelling porous enzyme-polymer matrices, monomers possessing functional groups other than (or in addition to) epoxide groups will be incorporated into the rigid, macroporous polymer during the polymerization reaction (i.e. aminostyrene). As in this embodiment, the bio-sensing enzyme could then be immobilized onto the polymer substrate using an appropriate

homobifunctional, heterobifunctional, trifunctional, and/or zero-length cross-linking agent.

In yet another embodiment of the rigid, non-swelling porous enzyme-polymer matrices, PhotoLink® (SurModics, Eden Prairie, MN) chemistry can be used to

5    immobilize the bio-sensing enzyme to the molded, porous, rigid polymer. In this embodiment, the polymer substrate need not possess any functional groups because the PhotoLink® chemistry reacts with carbon-hydrogen groups found in virtually every organic polymer.

10    C.    TYPICAL PROTOCOLS AND MATERIALS USEFUL IN THE
             MANUFACTURE OF ANALYTE SENSORS

The disclosure provided herein includes sensors and sensor designs that can be generated using combinations of various well known techniques. The disclosure further

15    provides methods for applying very thin enzyme coatings to these types of sensors as well as sensors produced by such processes. In this context, some embodiments of the invention include methods for making such sensors on a substrate according to art accepted processes. In certain embodiments, the substrate comprises a rigid and flat structure suitable for use in photolithographic mask and etch processes. In this regard,

20    the substrate typically defines an upper surface having a high degree of uniform flatness. A polished glass plate may be used to define the smooth upper surface. Alternative substrate materials include, for example, stainless steel, aluminum, and plastic materials such as delrin, etc. In other embodiments, the substrate is non-rigid and can be another layer of film or insulation that is used as a substrate, for example plastics such as

25    polyimides and the like.

An initial step in the methods of the invention typically includes the formation of a base layer of the sensor. The base layer can be disposed on the substrate by any desired means, for example by controlled spin coating. In addition, an adhesive may be used if there is not sufficient adhesion between the substrate layer and the base layer. A base

30    layer of insulative material is formed on the substrate, typically by applying the base layer material onto the substrate in liquid form and thereafter spinning the substrate to yield

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the base layer of thin, substantially uniform thickness. These steps are repeated to build

up the base layer of sufficient thickness, followed by a sequence of photolithographic

and/or chemical mask and etch steps to form the conductors discussed below. In an

illustrative form, the base layer comprises a thin film sheet of insulative material, such as

5      ceramic or polyimide substrate. The base layer can comprise an alumina substrate, a

polyimide substrate, a glass sheet, controlled pore glass, or a planarized plastic liquid

crystal polymer. The base layer may be derived from any material containing one or more

of a variety of elements including, but not limited to, carbon, nitrogen, oxygen, silicon,

sapphire, diamond, aluminum, copper, gallium, arsenic, lanthanum, neodymium,

10     strontium, titanium, yttrium, or combinations thereof. Additionally, the substrate may be

coated onto a solid support by a variety of methods well-known in the art including

chemical vapor deposition, physical vapor deposition, or spin-coating with materials such

as spin glasses, chalcogenides, graphite, silicon dioxide, organic synthetic polymers, and

the like.

15            The methods of the invention further include the generation of a conductive

layer having one or more sensing elements. Typically these sensing elements are

electrodes that are formed by one of the variety of methods known in the art such as

photoresist, etching and rinsing to define the geometry of the active electrodes. The

electrodes can then be made electrochemically active, for example by electrodeposition

20     of Pt black for the working and counter electrode, and silver followed by silver chloride

on the reference electrode. A sensor layer such as a sensor chemistry enzyme layer can

then be disposed on the sensing layer by electrochemical deposition or a method other

than electrochemical deposition such a spin coating, followed by vapor crosslinking, for

example with a dialdehyde (glutaraldehyde) or a carbodi-imide.

25            Electrodes of the invention can be formed from a wide variety of materials

known in the art. For example, the electrode may be made of a noble late transition

metals. Metals such as gold, platinum, silver, rhodium, iridium, ruthenium, palladium, or

osmium can be suitable in various embodiments of the invention. Other compositions

such as carbon or mercury can also be useful in certain sensor embodiments. Of these

30     metals, silver, gold, or platinum is typically used as a reference electrode metal. A silver

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electrode which is subsequently chloridized is typically used as the reference electrode. These metals can be deposited by any means known in the art, including the plasma deposition method cited, supra, or by an electroless method which may involve the deposition of a metal onto a previously metallized region when the substrate is dipped
5      into a solution containing a metal salt and a reducing agent. The electroless method proceeds as the reducing agent donates electrons to the conductive (metallized) surface with the concomitant reduction of the metal salt at the conductive surface. The result is  ·
a layer of adsorbed metal. (For additional discussions on electroless methods, see: Wise, E. M. Palladium: Recovery, Properties, and Uses, Academic Press, New York, New York
10     (1988); Wong, K. et al. Plating and Surface Finishing 1988, 75, 70-76; Matsuoka, M. et al. Ibid. 1988, 75, 102-106; and Pearlstein, F. "Electroless Plating," Modern Electroplating, Lowenheim, F. A., Ed., Wiley, New York, N.Y. (1974), Chapter 31.). Such a metal deposition process must yield a structure with good metal to metal adhesion and minimal surface contamination, however, to provide a catalytic metal electrode surface with a
15     high density of active sites. Such a high density of active sites is a property necessary for the efficient redox conversion of an electroactive species such as hydrogen peroxide.

       In an exemplary embodiment of the invention, the base layer is initially coated with a thin film conductive layer by electrode deposition, surface sputtering, or other suitable process step. In one embodiment this conductive layer may be provided as a
20     plurality of thin film conductive layers, such as an initial chrome-based layer suitable for chemical adhesion to a polyimide base layer followed by subsequent formation of thin film gold-based and chrome-based layers in sequence. In alternative embodiments, other electrode layer conformations or materials can be used. The conductive layer is then covered, in accordance with conventional photolithographic techniques, with a selected
25     photoresist coating, and a contact mask can be applied over the photoresist coating for suitable photoimaging. The contact mask typically includes one or more conductor trace patterns for appropriate exposure of the photoresist coating, followed by an etch step resulting in a plurality of conductive sensor traces remaining on the base layer. In an illustrative sensor construction designed for use as a subcutaneous glucose sensor, each
30     sensor trace can include three parallel sensor elements corresponding with three separate

electrodes such as a working electrode, a counter electrode and a reference electrode.

Portions of the conductive sensor layers are typically covered by an insulative cover layer, typically of a material such as a silicon polymer and/or a polyimide. The insulative cover layer can be applied in any desired manner. In an exemplary procedure,

5      the insulative cover layer is applied in a liquid layer over the sensor traces, after which the substrate is spun to distribute the liquid material as a thin film overlying the sensor traces and extending beyond the marginal edges of the sensor traces in sealed contact with the base layer. This liquid material can then be subjected to one or more suitable radiation and/or chemical and/or heat curing steps as are known in the art. In alternative

10     embodiments, the liquid material can be applied using spray techniques or any other desired means of application. Various insulative layer materials may be used such as photoimagable epoxyacrylate, with an illustrative material comprising a photoimagable polyimide available from OCG, Inc. of West Paterson, N.J., under the product number 7020.

15     As noted above, appropriate electrode chemistries defining the distal end electrodes can be applied to the sensor tips, optionally subsequent to exposure of the sensor tips through the openings. In an illustrative sensor embodiment having three electrodes for use as a glucose sensor, an enzyme (typically glucose oxidase) is provided within one of the openings, thus coating one of the sensor tips to define a working

20     electrode. One or both of the other electrodes can be provided with the same coating as the working electrode. Alternatively, the other two electrodes can be provided with other suitable chemistries, such as other enzymes, left uncoated, or provided with chemistries to define a reference electrode and a counter electrode for the electrochemical sensor.

25     A significant aspect of the present invention involves processes for making sensors having extremely thin coatings for electrode chemistries (e.g., enzyme coatings of less than 2 microns in thickness) with enhanced material properties. Methods for producing the extremely thin enzyme coatings of the invention include spin coating processes, dip and dry processes, low shear spraying processes, ink-jet printing processes,

30     silk screen processes and the like. As artisans can readily determine the thickness of an

enzyme coat applied by process of the art, they can readily identify those methods
capable of generating the extremely thin coatings of the invention. Typically, such
coatings are vapor crosslinked subsequent to their application. Surprisingly, sensors
produced by these processes have material properties that exceed those of sensors having
5     coatings produced by electrodeposition including enhanced longevity, linearity, regularity
as well as improved signal to noise ratios. In addition, embodiments of the invention
that utilize glucose oxidase coatings formed by such processes are designed to recycle
. hydrogen peroxide and improve the biocompatibility profiles of such sensors.

            While not being bound by a specific scientific theory, it is believed that the
10    surprising properties of sensors produced by such processes have enhanced
characteristics as compared to those generated by electrodeposition because
electrodeposition produces 3-5 micron thick enzyme layers in which only a fraction of
the reactive enzyme is able to access the analyte to be sensed. Moreover, in sensors
utilizing glucose oxidase, the thick coatings produced by electrodeposition may hinder
15    the ability of hydrogen peroxide generated at the reactive interface to reach the sensor
surface and thereby generate a signal. Moreover, hydrogen peroxide that is unable to
reach a sensor surface due to such thick coatings typically diffuses away from the sensor
into the environment in which the sensor is placed, thereby decreasing the
biocompatibility of such sensors. In addition, as glucose oxidase and albumin have
20    different isoelectric points, electrodeposition processes can result in a surface coating in
which an optimally determined ratio of enzyme to carrier protein is detrimentally altered
and further wherein the glucose oxidase and the carrier protein are not distributed in a
substantially uniform manner throughout the disposed enzyme layer. The thin coating
processes utilized to produce the sensors disclosed herein avoid these problems
25    associated with electrodeposition.

            Sensors generated by processes such as spin coating processes also avoid other
problems associated with electrodeposition, such as those pertaining to the material
stresses placed on the sensor during the electrodeposition process. In particular, the
process of electrodeposition is observed to produce mechanical stresses on the sensor,
30    for example mechanical stresses that result from tensile and/or compression forces. In

certain contexts, such mechanical stresses may result in sensors having coatings with some tendency to crack or delaminate. This is not observed in coatings disposed on sensor via spin coating or other low-stress processes. Consequently, yet another embodiment of the invention is a method of avoiding the electrodeposition influenced

5   cracking and or delamination of a coating on a sensor comprising applying the coating via a spin coating process.

Subsequent to treatment of the sensor elements, one or more additional functional coating or cover layers can then be applied by any one of a wide variety of methods known in the art, such as spraying, dipping, etc. Some embodiments of the

10  present invention include an analyte modulating layer deposited over the enzyme-containing layer. In addition to its use in modulating the amount of analyte(s) that contacts the active sensor surface, by utilizing an analyte limiting membrane layer, the problem of sensor fouling by extraneous materials is also obviated. As is known in the art, the thickness of the analyte modulating membrane layer can influence the amount of

15  analyte that reaches the active enzyme. Consequently, its application is typically carried out under defined processing conditions, and its dimensional thickness is closely controlled. Microfabrication of the underlying layers can be a factor which affects dimensional control over the analyte modulating membrane layer as well as exact the composition of the analyte limiting membrane layer material itself. In this regard, it has

20  been discovered that several types of copolymers, for example, a copolymer of a siloxane and a nonsiloxane moiety, are particularly useful. These materials can be microdispensed or spin-coated to a controlled thickness. Their final architecture may also be designed by patterning and photolithographic techniques in conformity with the other discrete structures described herein. Examples of these nonsiloxane-siloxane copolymers include,

25  but are not limited to, dimethylsiloxane-alkene oxide, tetramethyldisiloxane-divinylbenzene, tetramethyldisiloxane-ethylene, dimethylsiloxane-silphenylene, dimethylsiloxane-silphenylene oxide, dimethylsiloxane-a-methylstyrene, dimethylsiloxane-bisphenol A carbonate copolymers, or suitable combinations thereof. The percent by weight of the nonsiloxane component of the copolymer can be preselected to any useful

30  value but typically this proportion lies in the range of about 40-80 wt %. Among the

copolymers listed above, the dimethylsiloxane-bisphenol A carbonate copolymer which comprises 50-55 wt % of the nonsiloxane component is typical. These materials may be purchased from Petrarch Systems, Bristol, Pa. (USA) and are described in this company's products catalog. Other materials which may serve as analyte limiting membrane layers

5    include, but are not limited to, polyurethanes, cellulose acetate, cellulose nitrate, silicone rubber, or combinations of these materials including the siloxane nonsiloxane copolymer, where compatible.

In some embodiments of the invention, the sensor is made by methods which apply an analyte modulating layer that comprises a hydrophilic membrane coating which

10   can regulate the amount of analyte that can contact the enzyme of the sensor layer. For example, the cover layer that is added to the glucose sensors of the invention can comprise a glucose limiting membrane, which regulates the amount of glucose that contacts glucose oxidase enzyme layer on an electrode. Such glucose limiting membranes can be made from a wide variety of materials known to be suitable for such

15   purposes, e.g., silicones such as polydimethyl siloxane and the like, polyurethanes, cellulose acetates, Nafion, polyester sulfonic acids (e.g. Kodak AQ), hydrogels or any other membrane known to those skilled in the art that is suitable for such purposes. In certain embodiments of the invention pertaining to sensors having hydrogen peroxide recycling capabilities, the membrane layer that is disposed on the glucose oxidase enzyme

20   layer functions to inhibit the release of hydrogen peroxide into the environment in which the sensor is placed and to facilitate the contact between the hydrogen peroxide molecules and the electrode sensing elements.

In some embodiments of the methods of invention, an adhesion promoter layer is disposed between a cover layer (e.g. an analyte modulating membrane layer) and a

25   sensor chemistry layer in order to facilitate their contact and is selected for its ability to increase the stability of the sensor apparatus. As noted herein, compositions of the adhesion promoter layer are selected to provide a number of desirable characteristics in addition to an ability to provide sensor stability. For example, some compositions for use in the adhesion promoter layer are selected to play a role in interference rejection as

30   well as to control mass transfer of the desired analyte. The adhesion promoter layer can

be made from any one of a wide variety of materials known in the art to facilitate the bonding between such layers and can be applied by any one of a wide variety of methods known in the art. Typically, the adhesion promoter layer comprises a silane compound such as γ-aminopropyltrimethoxysilane. In certain embodiments of the invention, the

5      adhesion promoting layer and/or the analyte modulating layer comprises an agent selected for its ability to crosslink a siloxane moiety present in a proximal. In other embodiments of the invention, the adhesion promoting layer and/or the analyte modulating layer comprises an agent selected for its ability to crosslink an amine or carboxyl moiety of a protein present in a proximal layer. In an optional embodiment, the

10     AP layer further comprises Polydimethyl Siloxane (PDMS), a polymer typically present in analyte modulating layers such as a glucose limiting membrane. In illustrative embodiments the formulation comprises 0.5-20% PDMS, typically 5-15% PDMS, and most typically 10% PDMS. The addition of PDMS to the AP layer can be advantageous in contexts where it diminishes the possibility of holes or gaps occurring in the AP layer

15     as the sensor is manufactured.

       As noted above, a coupling reagent commonly used for promoting adhesion between sensor layers is γ-aminopropyltrimethoxysilane. The silane compound is usually mixed with a suitable solvent to form a liquid mixture. The liquid mixture can then be applied or established on the wafer or planar sensing device by any number of ways

20     including, but not limited to, spin-coating, dip-coating, spray-coating, and microdispensing. The microdispensing process can be carried out as an automated process in which microspots of material are dispensed at multiple preselected areas of the device. In addition, photolithographic techniques such as "lift-off" or using a photoresist cap may be used to localize and define the geometry of the resulting permselective film

25     (i.e. a film having a selective permeability). Solvents suitable for use in forming the silane mixtures include aqueous as well as water-miscible organic solvents, and mixtures thereof. Alcoholic water-miscible organic solvents and aqueous mixtures thereof are particularly useful. These solvent mixtures may further comprise nonionic surfactants, such as polyethylene glycols (PEG) having a for example a molecular weight in the range

30     of about 200 to about 6,000. The addition of these surfactants to the liquid mixtures, at

a concentration of about 0.005 to about 0.2 g/dL of the mixture, aids in planarizing the resulting thin films. Also, plasma treatment of the wafer surface prior to the application of the silane reagent can provide a modified surface which promotes a more planar established layer. Water-immiscible organic solvents may also be used in preparing

5     solutions of the silane compound. Examples of these organic solvents include, but are not limited to, diphenylether, benzene, toluene, methylene chloride, dichloroethane, trichloroethane, tetrachloroethane, chlorobenzene, dichlorobenzene, or mixtures thereof. When protic solvents or mixtures thereof are used, the water eventually causes hydrolysis of the alkoxy groups to yield organosilicon hydroxides (especially when n=1) which

10    condense to form poly(organosiloxanes). These hydrolyzed silane reagents are also able to condense with polar groups, such as hydroxyls, which may be present on the substrate surface. When aprotic solvents are used, atmospheric moisture may be sufficient to hydrolyze the alkoxy groups present initially on the silane reagent. The R' group of the silane compound (where n=1 or 2) is chosen to be functionally compatible with the

15    additional layers which are subsequently applied. The R' group usually contains a terminal amine group useful for the covalent attachment of an enzyme to the substrate surface (a compound, such as glutaraldehyde, for example, may be used as a linking agent as described by Murakami, T. et al., Analytical Letters 1986, 19, 1973-86).

      Like certain other coating layers of the sensor, the adhesion promoter layer can

20    be subjected to one or more suitable radiation and/or chemical and/or heat curing steps as are known in the art. In alternative embodiments, the enzyme layer can be sufficiently crosslinked or otherwise prepared to allow the membrane cover layer to be disposed in direct contact with the sensor chemistry layer in the absence of an adhesion promoter layer.

25    An illustrative embodiment of the invention is a method of making a sensor by providing a base layer, forming a sensor layer on the base layer, spin coating an enzyme layer on the sensor layer and then forming an analyte contacting layer (e.g. an analyte modulating layer such as a glucose limiting membrane) on the sensor, wherein the analyte contacting layer regulates the amount of analyte that can contact the enzyme layer. In

30    some methods, the enzyme layer is vapor crosslinked on the sensor layer. In a typical

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embodiment of the invention, the sensor layer is formed to include at least one working electrode and at least one counter electrode. In certain embodiments, the enzyme layer is formed on at least a portion of the working electrode and at least a portion of the counter electrode. Typically, the enzyme layer that is formed on the sensor layer is less

5      than 2, 1, 0.5, 0.25 or 0.1 microns in thickness. Typically, the enzyme layer comprises one or more enzymes such as glucose oxidase, glucose dehydrogenase, lactate oxidase, hexokinase or lactate dehydrogenase and/or like enzymes. In a specific method, the enzyme layer comprises glucose oxidase that is stabilized by coating it on the sensor layer in combination with a carrier protein in a fixed ratio. Typically the carrier protein is

10     albumin. Typically such methods include the step of forming an adhesion promoter layer disposed between the glucose oxidase layer and the analyte contacting layer. Optionally, the adhesion promoter layer is subjected to a curing process prior to the formation of the analyte contacting layer.

A related embodiment of the invention is a method of making a glucose sensor

15     by providing a base layer, forming a sensor layer on the base layer that includes at least one working electrode and at least one counter electrode, forming a glucose oxidase layer on the sensor layer by a spin coating process (a layer which is typically stabilized by combining the glucose oxidase with albumin in a fixed ratio), wherein the glucose oxidase layer coats at least a portion of the working electrode and at least a portion of the

20     counter electrode, and then forming a glucose limiting layer on the glucose sensor so as to regulate the amount of glucose that can contact the glucose oxidase layer. In such processes, the glucose oxidase layer that is formed on the sensor layer is typically less than 2, 1, 0.5, 0.25 or 0.1 microns in thickness. Typically, the glucose oxidase coating is vapor crosslinked on the sensor layer. Optionally, the glucose oxidase coating covers the

25     entire sensor layer. In certain embodiments of the invention, an adhesion promoter layer is disposed between the glucose oxidase layer and the analyte contacting layer. In certain embodiments of the invention, the analyte sensor further comprises one or more cover layers which are typically electrically insulating protective layers (see, e.g. element 106 in Figure 2). Typically, such cover layers are disposed on at least a portion of the analyte

30     modulating layer.

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The finished sensors produced by such processes are typically quickly and easily removed from a supporting substrate (if one is used), for example, by cutting along a line surrounding each sensor on the substrate. The cutting step can use methods typically used in this art such as those that include a UV laser cutting device that is used to cut

5   through the base and cover layers and the functional coating layers along a line surrounding or circumscribing each sensor, typically in at least slight outward spaced relation from the conductive elements so that the sufficient interconnected base and cover layer material remains to seal the side edges of the finished sensor. In addition, dicing techniques typically used to cut ceramic substrates can be used with the

10  appropriate sensor embodiments. Since the base layer is typically not physically attached or only minimally adhered directly to the underlying supporting substrate, the sensors can be lifted quickly and easily from the supporting substrate, without significant further processing steps or potential damage due to stresses incurred by physically pulling or peeling attached sensors from the supporting substrate. The supporting substrate can

15  thereafter be cleaned and reused, or otherwise discarded. The functional coating layer(s) can be applied either before or after other sensor components are removed from the supporting substrate (e.g., by cutting).


D.      CYCLIC PLATING OF METALS TO PRODUCE BIOSENSOR
20         ELECTRODES

Another illustrative embodiment of the invention is a method of making a metallic electrode useful in biosensors and electrodes made by such methods. These methods for making a metallic electrode include electrodepositing a plurality of metal

25  layers that comprise the electrode using cycles of differing electroplating conditions. Typically, the method comprises a first cycle of electroplating where a metal is electrodeposited onto a substrate under a first set of conditions selected to produce a first metal layer having a first surface area and a first adhesion strength between the substrate and the first metal layer. The method then involves a second cycle of

30  electroplating where a metal composition is then electrodeposited onto the first metal layer under a second set of conditions selected to produce a second metal layer having a

second surface area and a second adhesion strength between the first metal layer and the second metal layer. In this method, the first and second set of conditions are selected to produce a second metal layer having a second surface area that is greater (e.g. at least 5%, 10%, 15%, 20%, 25%, or 30% greater) than the first surface area of the first metal layer

5      produced by the first set of conditions and a second metal layer having an adhesion with the first metal layer that is greater (e.g. at least 5%, 10%, 15%, 20%, 25%, or 30% greater) than the adhesion between the first metal layer and the substrate produced by the first set of conditions. Optionally, the method further comprises additional cycles of electroplating. In one such example, the method comprises a third cycle of

10     electroplating where a metal composition is electrodeposited onto the second layer under a third set of conditions selected to produce a third metal layer having a third surface area. Typically, the second and third set of conditions are selected to produce a third metal layer having a greater density (e.g. at least 5%, 10%, 15%, 20%, 25%, or 30% greater) than the density of the second metal layer. In certain embodiments of the

15     invention, the third set of conditions produces a third metal layer having a third surface area that is less than the second surface area of the second metal layer produced by the second set of conditions.

These embodiments of the invention can be used in a variety of contexts. In certain embodiments of the invention, the substrate does not comprise platinum and

20     comprises a polymer or another metal such as gold. Optionally, the substrate comprises a geometric feature selected to increase the surface area of the electroplated metal composition. In certain embodiments of the invention, the metal composition is electroplated on to a porous substrate. Optionally, the substrate comprises a planar surface and an edge or lip at the boundary of the planar surface and the cycles of

25     differing electroplating conditions further inhibit an uneven deposition of the metal layer electrodeposited onto the planar surface and the edge or lip at the boundary of the planar surface. Typically, the uneven deposition of the metal layer that is inhibited is a greater deposition of metal on the edge or lip at the boundary of the planar surface relative to the deposition of metal on the planar surface. Optionally, the electroplated metal

30     composition comprises platinum. Optionally, the surface area of the third layer is at least

160, 170 or 180 times the geometric area of the third layer and is typically 230 – 260 times the geometric area of the third layer.

A variety of protocols and conditions for making electrodes of the invention via electroplating have been well know in the art for over 30 years. For example, Feltham

5     and Spiro in Chemical Reviews, 1971, Vol. 71, No. 2, pp: 177-193 which is incorporated herein by reference teach illustrative protocols and conditions for making electrodes via electroplating and further teach how these conditions (e.g. current density) can be controlled to affect the material properties of the electrodeposited metal layers, for example their density and roughness (factors which correlate to surface area).

10    Electrodeposition conditions and the ways in which these conditions can be controlled to affect the material properties of electrodeposited metal layers are also described for example in U.S. Patent Nos. 4,153,521, 4,280,882, 4,285,784, 4,358,352, 4,427,502, 4,879,013, 4,919,768, 5,310,475, 6,139,711 and 6,596,149, the contents of which are incorporated herein by reference.   See also, Modern Electroplating (4th edition), M.

15    Schlesinger and M. Paunovic (editors), Wiley, New York, 2000. Fundamentals of Electrochemical Deposition, M. Paunovic and M. Schlesinger, Wiley, New York, 1998, the contents of which are incorporated herein by reference.

In certain protocols known in the art, platinum black is electroplated onto a sensor electrode (e.g. as is used in glucose oxidase based glucose sensors) at relatively

20    high current densities to cause a very rough platinum surface to develop. Using for example the recommended platinizing solutions and procedures described at page 193 of the Feltham and Spiro article (Chemical Reviews, 1971, Vol. 71, No. 2), at a plating current density of 186 μA/sq. mm for 120 seconds, the resultant surface area ranges from 160 to 180 times the geometric surface area. Though high surface areas are

25    achieved by this set of conditions, in certain circumstances, such conditions can produce electrodes having somewhat problematic material properties. For example, using this type of current density protocol under controlled conditions (e.g. solution concentrations), excessive metal (e.g. platinum) "growth" may be observed along the periphery of the electrode made by electrodepositing layers of a metal(s) onto a substrate

30    having a planar surface and the edge or lip at the boundary of the planar surface. This

"growth" is for example the result of an uneven deposition of the metal layer electrodeposited onto the planar surface and the edge or lip at the boundary of the planar surface. In addition to this excessive growth, using this current density under controlled conditions can result in a metal layer that is fragile and further has poor adhesion with

5　　the underlying substrate (e.g. a fragile platinum layer that exhibits a marginal adhesion to an underlying gold surface).

In illustrative embodiments of the invention using for example the recommended platinizing solutions and procedures described on page 193 of the Feltham and Spiro article, cyclic plating conditions are used that vary the applied current density during the

10　　plating process. Specific illustrative conditions are provided in Tables 1 and 2 below. In illustrative cycles of conditions that vary the current density and time in a defined solution of electroplated metals, a low current density is first applied for 30 to 120 seconds (90 uA/sq. mm). A higher current is then applied for 30 to 120 seconds (140 uA/sq. mm). Finally, a low current density can be again applied for 30 to 120 seconds

15　　(90 uA/sq. mm). Electrodes made by such specific combinations of cyclic plating conditions exhibit a number of surprising and beneficial material properties. For example, in this illustrative embodiment, a first low current density cycle condition results in a smooth platinum layer, one that exhibits a strong adhesion to a substrate and a corresponding good resistance to abrasion (e.g. as measured by an art accepted

20　　abrasion test such as a DIN, TBS or Taber abrasion test method). In certain embodiments of the invention, the first metal layer electroplated under a first set of conditions therefore exhibits a resistance to abrasion from the substrate that is greater than the resistance to abrasion exhibited by a metal layer electroplated to the substrate under the second set of conditions. The cyclic plating conditions in this cycle therefore

25　　produce a metal layer that has the beneficial material property of good adherence between elements (e.g. the substrate and the first metal layer). Once the first layer is "anchored" by this first set of conditions, a second higher current density cycle is used to produce a further layer on this base layer on that exhibits a roughness that is correlated with a high surface area, a desirable property for electrodes that are for

30　　example coated with enzyme layer, such as an enzyme capable of reacting with and/or

producing a molecule whose change in concentration can be measured by measuring a change in the current at the electrode (e.g. glucose oxidase). At the same time, electrodes made using these first and second conditions produce an electrodeposited metal layer where excessive "edge" growth (a factor that can lead to instability of electrode

5     structures) is inhibited. In certain embodiments of the invention, a further low density cycle plating cycle is then used to plate an additional metal layer that essentially hardens the rough layer of metal created in the second cycle (e.g. by making the layer more dense). Unexpectedly, electrodes made by this cycle of plating conditions exhibit a constellation of desirable material properties including: (1) a good adhesion to the

10    substrate (e.g. resulting from the conditions of cycle 1); (2) a desirable amount or degree of surface area (e.g. resulting from the conditions of cycle 2); and (3) a desirable hardness or density for final surface area of the electrode (e.g. resulting from the conditions of cycle 3). Alone and especially in combination, all of these material properties contribute to the creation of a stabilized electrode structure. Electrodes plated in this fashion

15.   exhibit good adhesion to a gold substrate as well as good abrasion resistance. Furthermore, the resultant surface area ranges from 230-260 times the geometric surface area.

        Another embodiment of the invention is an implantable biosensor comprising an electrode comprising a plurality of electrodeposited metal layers including a first metal

20    layer having a first surface area and a first adhesion strength with a substrate on which the first layer is electrodeposited and a second metal layer deposited on the first metal layer, the second metal layer having a second surface area and a second adhesion strength with the first layer on which the second layer is electrodeposited, wherein the second surface area is greater than the first surface area and the second adhesion strength is

25    greater than the first adhesion strength; and further an enzyme layer disposed on the electrode, wherein an enzyme in the enzyme layer is capable of reacting with and/or producing a molecule whose change in concentration can be measured by measuring a change in the current at the electrode. In certain embodiments of the invention, the electrode comprises a third metal layer deposited on the second metal layer, wherein the

30    third metal layer has a greater density than the density of the second metal layer.

Optionally the enzyme layer comprises glucose oxidase or lactate oxidase.

Yet another embodiment of the invention is an electrode comprising a plurality of electrodeposited metal layers comprising a first metal layer having a first surface area; a second metal layer deposited on the first metal layer, the second metal layer having a second surface area that is greater than the first surface area of the first metal layer; and a third metal layer deposited on the second metal layer, the third metal layer having a greater density than the density of the second metal layer. Optionally the substrate comprises a geometric feature selected to increase the surface area of the electrodeposited metal composition.

E.      MICRO-FABRICATED POLY(DIMETHYL SILOXANE) MEMBRANE
        FOR USE AS THE PERMSELECTIVE SENSOR LAYER

As noted above, certain sensor embodiments achieve their bio-specificity through immobilized enzymes such as glucose oxidase (GOx) or lactate dehydrogenase (LDH), which consume oxygen along with glucose or lactate (the sensor analytes) as co-reactants. To minimize the sensitivity of the reaction rate to the oxygen concentration in such sensors, a molar excess of oxygen is required. However, normal physiologic conditions are such that glucose (~5 mM) and lactate (~1 mM) are almost always found in molar excess of oxygen (~0.05 mM). Hence, most existing sensor designs employ a membrane that is significantly more permeable to oxygen than it is to the analyte. These permselective membranes usually contain poly(dimethylsiloxane) (PDMS), as it is biocompatible and typically possesses an unusually high permeability to oxygen and virtually no permeability to analytes such as glucose or lactate. Limited analyte permeability is typically imparted upon the PDMS-based material either by co-polymerizing PDMS with a hydrophilic polymer (i.e. Jeffamine®) or by cutting a macroscopic "window" into a tube or sheet of PDMS.

Copolymer-type permselective membranes have successfully been used in clinically approved short-term (less than 1-week) subcutaneous glucose sensors (e.g. Continuous Glucose Monitoring Systems ("CGMS) and/or a Telemetered Glucose Monitoring Systems ("TGMS"). This type of membrane simply requires analyte to

diffuse across its thickness, which is optimal for sensor linearity and response time. However, due to the poor long-term (i.e. 1-year) *in vivo* stability of hydrophilic polymers, the feasibility of using such a permselective membrane in a long-term implantable sensor remains in doubt. Meanwhile, macroscopic window-type permselective membranes offer

5      excellent long-term stability, *in vivo*. However, this type of membrane requires analyte to diffuse in an extra macroscopic dimension, which can negatively impact sensor linearity as well as response time. Embodiments of the invention produce a permselective membrane fashioned from micro-fabricated PDMS that possesses the inherent advantages of both co-polymer and window-type biosensor membranes. While others

10     have constructed PDMS microstructures through the casting of PDMS pre-polymers into complementary micro-fabricated relief patterns (e.g. Kumar et al., 1994, *Langmuir* 10: 1498-1511; Dapprich, 2003, US 6,585,939), no one has previously described the use of micro-fabricated PDMS as the permselective membrane in an enzymatic electrochemical biosensor.

15          In one embodiment of the invention, photolithography, lithographic molding, thick-film printing, plasma polymerization (with or without shadow-masking), or discrete nano-dispensing can be used to micro-pattern a curable PDMS functionalized derivative, co-polymer, or mixture thereof onto a pre-cast immuno-isolation membrane. Vacuum or a pressure gradient may or may not be applied to promote the filling of the pores of

20     the immuno-isolation membrane. Composite membranes fashioned in such a manner can possess morphologies that are layered, pore-filled, or some combination thereof.

           In another embodiment of the invention, a curable PDMS derivative, co-polymer, or mixture thereof can be micro-patterned onto a temporary substrate using the aforementioned techniques. In the final sensor assembly, the stand-alone part may be

25     used with a phase-inversion membrane ("PIM") that may either be cast as a separate part or on top of the PDMS (filling its pores). Various methods for promoting adhesion may be employed by individuals skilled in the art.

           In an alternate embodiment, a curable PDMS derivative, co-polymer, or mixture thereof can be micro-patterned directly onto the sensor assembly, using the techniques

30     described above. A PIM may be placed or cast on top of the micro-patterned PDMS.

Various methods for promoting adhesion may be employed by individuals skilled in the art.

In another embodiment, a laser can be used to micro-machine holes (0.1-1000 microns) into a formed piece of PDMS co-polymer (or another polymeric composition such as "silicone rubber") to form a microporous membrane. Again, the PDMS membrane may be used with or without a PIM in the final sensor assembly. Various methods for promoting adhesion can be employed by individuals skilled in the art.

Illustrative chemically active groups that can be used to functionalize the PDMS and/or PDMS co-polymer include, but are not limited to: methacrylates, acrylates, vinyls, hydrides, silanols, alkoxys, amines, epoxides, carbinols, and mercaptos. Examples of monomers that can be used to make the PDMS copolymer include, but are not limited to: phenylmethyl-, vinylmethyl-, diethyl-, methacryloxypropylmethyl-, acryloxypropylmethyl-, and alkylmethyl-siloxanes. The immuno-isolation membrane can be pre-cast from a biocompatible polymer such as poly(acrylonitrile-vinyl chloride) (PAN-PVC), for example using a phase-inversion process that can be optimized by individuals well-trained in the fields of biomaterials and polymer chemistry. The casting of the phase-inversion membrane (PIM) and the micro-patterning of the PDMS can be performed on the sensor assembly itself or on a temporary substrate such as a glass slide or silicon wafer (e.g. to form a separate part). A micro-patterned temporary substrate can also be used to create micro-wells in the PIM into which the PDMS could be patterned. In addition, individuals skilled in the art can employ various chemicals and techniques for promoting adhesion between the PDMS and the PIM. Examples include, but are not limited to the use of: functionalized PDMS derivatives, silanes, silane esters, functionalized silane esters, cross-linking agents, reactive polymer coatings (i.e. Lahann et al., 2003, *Anal. Chem.* 75: 2117-2122), plasma treatment, plasma polymerization, shadow masking, and chemical vapor deposition.

The permselective membranes containing poly(dimethylsiloxane) provide a variety of embodiments of the invention. One embodiment of the invention is a method of making a membrane for use with an implantable analyte sensor by forming a first layer of material comprising a biocompatible polymer composition that is impermeable to

immunoglobulins yet permeable to oxygen, glucose and lactate, and then coupling the

first layer to a second layer comprising functionalized poly(dimethyl siloxane),

functionalized poly(dimethyl siloxane) copolymer or a mixture of functionalized

poly(dimethyl siloxane) and functionalized poly(dimethyl siloxane) copolymer so that a

5     membrane for use with an implantable analyte is made. In certain embodiments of the

invention, a first layer of material comprising a biocompatible polymer composition that

is impermeable to immunoglobulins is termed a "immuno-isolation membrane". The

membrane made by this method is more permeable to oxygen than it is to compounds

having a higher molecular weight such as glucose and/or lactate. Composite membranes

10    fashioned in such a manner can be made to possess a variety of morphologies, including

those that are layered, pore-filled, or some combination thereof.

     Illustrative chemically active groups that can be used to functionalize the PDMS

and/or PDMS co-polymer include, but are not limited to: methacrylates, acrylates, vinyls,

hydrides, silanols, alkoxys, amines, epoxides, carbinols, and mercaptos. Examples of

15    monomers that can be used to make the PDMS copolymer include, but are not limited

to: phenylmethyl-, vinylmethyl-, diethyl-, methacryloxypropylmethyl-,

acryloxypropylmethyl-, and alkylmethyl-siloxanes. The other layer (e.g. the immuno-

isolation membrane) can be pre-cast from a biocompatible polymer such as

poly(acrylonitrile-vinyl chloride) (PAN-PVC), for example using a phase-inversion

20    process that can be optimized by individuals well-trained in the fields of biomaterials and

polymer chemistry. The casting of the phase-inversion membrane (PIM) and the micro-

patterning of the PDMS can be performed on the sensor assembly itself or on a

temporary substrate such as a glass slide or silicon wafer (e.g. to form a separate part). A

micro-patterned temporary substrate can also be used to create micro-wells in the PIM

25    into which the PDMS could be patterned. While certain embodiments of the invention

include analyte sensors with composite membranes, the PDMS membrane may be used

with or without a PIM in the final sensor assembly.

     Embodiments of these membranes can be made using a variety of well-known

techniques. For example, in one illustrative embodiment, photolithography, lithographic

30    molding, thick-film printing, plasma polymerization (with or without shadow-masking),

or discrete nano-dispensing can be used to micro-pattern a curable PDMS functionalized derivative, co-polymer, or mixture thereof onto a pre-cast immuno-isolation membrane. In another embodiment of the invention, a curable PDMS derivative, co-polymer, or mixture thereof can be micro-patterned onto a temporary substrate using the described

5     techniques. In the final sensor assembly, the stand-alone part may be used with a phase-inversion membrane that may either be cast as a separate part or on top of the PDMS (filling its pores). In an alternate embodiment, a curable PDMS derivative, co-polymer, or mixture thereof can be micro-patterned directly onto the sensor assembly, using the techniques described above. A PIM may be placed or cast on top of the micro-patterned

10    PDMS.

Optionally, an adhesive layer disposed between the first and second layers of the membrane to promote adhesion between the first and second layers (as well as any other sensor layer where such an adhesive layer is appropriate). Various methods for promoting adhesion between the layers of the membrane may be employed by

15    individuals skilled in the art. For example, a micro-patterned temporary substrate can also be used to create micro-wells in the PIM into which the PDMS could be patterned. In addition, individuals skilled in the art can employ various chemicals and techniques for promoting adhesion between the PDMS and the PIM. Examples include, but are not limited to the use of functionalized PDMS derivatives, silanes, silane esters,

20    functionalized silane esters, cross-linking agents, reactive polymer coatings (see, e.g., Lahann et al., 2003, *Anal. Chem.* 75: 2117-2122), plasma treatment, plasma polymerization, shadow masking, and chemical vapor deposition.

In certain embodiments of the invention, the analyte sensor membrane can include additional layers having other compositions used in the manufacture of analyte

25    sensors such as those described herein. In addition, in some embodiments of the invention, the first layer and/or the second layer of the membrane is constructed to include a plurality of pores. For example a laser can be used to micro-machine holes (e.g. of about 0.1 to about 1000 microns in size) into a formed piece of PDMS co-polymer (or another polymeric composition such as "silicone rubber") to form a microporous

30    membrane. In some embodiments of the invention, at least one of the plurality of pores

disposed in the second layer contains functionalized poly(dimethyl siloxane), functionalized poly(dimethyl siloxane) copolymer or a mixture of functionalized poly(dimethyl siloxane) and functionalized poly(dimethyl siloxane) copolymer of the first layer.

5          A related embodiment of the invention is a membrane made by the disclosed methods. One such embodiment of the invention is a membrane for use with an implantable analyte sensor which includes a first layer comprising a biocompatible polymer composition that is impermeable to immunoglobulins, yet permeable to oxygen, glucose and lactate; and a second layer coupled to the first layer comprising

10    functionalized poly(dimethyl siloxane), functionalized poly(dimethyl siloxane) copolymer or a mixture of functionalized poly(dimethyl siloxane) and functionalized poly(dimethyl siloxane) copolymer wherein the membrane is more permeable to oxygen than glucose and/or lactate. In certain embodiments of the invention, the first and/or the second layers in the membrane comprises a plurality of pores. In certain embodiments of the

15    invention, an adhesive layer disposed between the first and second layers, wherein the adhesive layer promotes adhesion between the first and second layers. Optionally, at least one of the plurality of pores disposed in the second layer contains functionalized poly(dimethyl siloxane), functionalized poly(dimethyl siloxane) copolymer or a mixture of functionalized poly(dimethyl siloxane) and functionalized poly(dimethyl siloxane)

20    copolymer of the second layer. Yet another embodiment of the invention is an analyte sensor having a membrane disclosed above, for example an analyte sensor having a membrane made according the described methods. A related embodiment is a method of making an analyte sensor having such a membrane.

          Another embodiment of the invention is a membrane for use with an implantable

25    analyte sensor, the membrane including a first layer comprising a biocompatible polymer composition that is impermeable to immunoglobulins, yet permeable to oxygen, glucose and lactate; and a second layer coupled to the first layer comprising functionalized poly(dimethyl siloxane), functionalized poly(dimethyl siloxane) copolymer or a mixture of functionalized poly(dimethyl siloxane) and functionalized poly(dimethyl siloxane)

30    copolymer. In this embodiment of the invention, the membrane is typically more

permeable to oxygen than glucose and/or lactate.

Optionally in this membrane for use with an implantable analyte sensor the first layer and/or the second layer comprises a plurality of pores disposed therein. In certain embodiments of the invention, at least one of the plurality of pores disposed in the

5   second layer contains functionalized poly(dimethyl siloxane), functionalized poly(dimethyl siloxane) copolymer or a mixture of functionalized poly(dimethyl siloxane) and functionalized poly(dimethyl siloxane) copolymer of the second layer. In some embodiments of the invention, an adhesive layer can be disposed between the first and second layers, wherein the adhesive layer promotes adhesion between the first and

10  second layers.

A related embodiment of the invention is a method of making a membrane for use with an implantable analyte sensor generating a first layer comprising functionalized poly(dimethyl siloxane), functionalized poly(dimethyl siloxane) copolymer or a mixture of functionalized poly(dimethyl siloxane) and functionalized poly(dimethyl siloxane)

15  copolymer; generating a second layer coupled to the first layer comprising a biocompatible polymer composition that is: impermeable to immunoglobulins; and permeable to oxygen, glucose and lactate so that a membrane is made that is more permeable to oxygen than glucose and/or lactate. Optionally in this method, the first layer and/or the second layer can be made to comprise a plurality of pores disposed

20  therein. In certain embodiments of the invention, at least one of the plurality of pores disposed in the second layer is made to contain functionalized poly(dimethyl siloxane), functionalized poly(dimethyl siloxane) copolymer or a mixture of functionalized poly(dimethyl siloxane) and functionalized poly(dimethyl siloxane) copolymer of the second layer. In some embodiments of the method, an adhesive layer can be disposed

25  between the first and second layers.

F.   MICROFABRICATION OF METALLIC MOLDS

A variety of methods for the microfabrication of polymerized compositions such as poly(dimethylsiloxane) or "PDMS" have been developed in recent years and are now

30  commonly used for the construction of devices such as MEMS (micro-electromechanical

96

systems) as well as in the micro-patterning of self-assembled monolayers (e.g. "soft lithography"). Typically, these methods involve the fabrication of a mold that is then filled with a polymerizable composition (e.g. a PDMS pre-polymer), which is cured (polymerized) and then released to yield a microfabricated PDMS element.

5      The molds used in these procedures are usually fabricated using one of two different approaches. In the first such approach, the negative photoresist is coated, patterned via photolithography, and developed on a base substrate. In the second such approach, silicon wafers are etched to form a relief pattern. In this context, the fabrication of molds with small, high aspect ratio features remains a significant challenge.

10    For example, molds with these extreme geometries typically have poor mechanical properties and can for example detach from the underlying substrate during polymer release.

      Mathematical modeling predicts that a layer of microporous PDMS with a high aspect ratio can be used as the permselective membrane of the types used for example in

15    enzymatic electrochemical glucose sensors. This mathematical modeling predicts that a sensor having such a membrane will exhibit a fast, linear response to glucose. Moreover, the well-known long-term stability of PDMS *in vivo* makes a permselective membrane attractive for use in a long-term implantable sensor such as the LTGS. Clearly, the microfabrication of a mold with small, high aspect ratio features that possess sufficient

20    mechanical strength to withstand PDMS release is highly desirable. In this context, embodiments of the invention disclosed herein include novel microfabrication methods that produce molds with mechanically robust features that are smaller in size and/or possess higher aspect ratios than those that can be produced through methods previously described in the art.

25    To form the layered substrate, a base substrate formed from a material such as but not limited to glass, silicon, silicon nitride, or aluminum oxide is coated by a process such as sputtering with a conductive material such as but not limited to gold, silver, platinum, copper, or chrome. The base substrate may be pre-coated with a layer of chrome or titanium in order to promote adhesion of the top conductive layer. A positive

30    photoresist such as AZ 4620 can then be applied by spin coating or by other methods

familiar to those skilled in the art of microfabrication. After pre-baking, a sacrificial thin film of a metal such as chrome can be sputtered onto the layer of positive photo-resist. A negative photoresist such as SU-8 can be applied by spin coating or by other methods familiar to those skilled in the art. The layered substrate can be obtained through

5    standard photolithography and subsequent development of the negative resist. Chrome etch or another appropriate etchant can be used to remove the areas of the sacrificial metal layer exposed by the development of the negative resist. The substrate can then be re-exposed to UV light, with or without the use of a photomask. The positive resist can then be developed using an appropriate developing solvent, which can be selected by

10   individuals skilled in the art. The resulting substrate can be electroplated with a conductive material such as, but not limited to gold, silver, platinum, copper, or chrome. The positive resist, sacrificial metal layer, and the negative resist can be removed from the substrate by exposing it to acetone or another solvent that can be selected by those skilled in the art. If deemed necessary, other solvents and/or etchants such as chrome

15   etch and negative resist strip can also be selected and applied by those skilled in the art. The resulting mold can be used repeatedly to microfabricate PDMS and other elastomers. The mold can also be used to microfabricate inelastic/hard materials in embodiments of the invention where the electroplated material can be removed by a chemical etchant or electrochemical oxidation.

20       One embodiment of the invention is a mold made by the methods described above. A related embodiment of the invention is a mold for forming a polymerized composition having a predetermined geometry comprising a metallic substrate capable of containing a polymerizable composition, where the polymerized composition produced by the mold has an aspect ratio geometry selected to facilitate the selective diffusion or

25   migration of a molecule involved in the sensing process. Another embodiment of the invention is a mold for forming a polymerized composition having a predetermined geometry comprising a metallic substrate capable of containing a polymerizable composition, where the polymerized composition produced by the mold is between about 10 and 2000 microns in thickness. Another embodiment of the invention is a

30   mold for forming a polymerized composition having a predetermined geometry

comprising a metallic substrate capable of containing a polymerizable composition, where the mold has sufficient mechanical strength to withstand release of a polymerized poly(dimethylsiloxane) composition without breaking. In certain embodiments of the invention, the mold has a two or more of these features.

5       One embodiment of the invention is a method of making a mold for forming a polymerized composition of a predetermined geometry by providing a base substrate; disposing a conductive layer on to (at least a portion) the base substrate; disposing a positive photoresist layer on to the conductive layer; disposing a sacrificial metal layer on to the positive photoresist layer; disposing a negative photoresist layer on to the

10      sacrificial metal layer; developing the negative photoresist layer via UV photolithography (with or without the use of a photomask); removing the areas of the sacrificial metal layer exposed by the development of the negative resist layer using an etchant; exposing these components to UV photolithography; developing the positive photoresist layer via a developing solvent; electroplating these components with a layer of conductive material;

15      and removing the positive photoresist layer, the sacrificial metal layer, and the negative photoresist layer from the so layered substrate using a solvent so that the mold is made. Typically, the mold made by the method can be used repeatedly. Other embodiments of the invention include a polymerized composition layer made using the described molds as well as analyte sensor including a polymerized composition layer made using the

20      described molds.

        In embodiments of the invention, the base substrate can be formed from a wide variety of materials such as glass, silicon, silicon nitride, aluminum oxide or the like. In certain embodiments of the invention the conductive material is disposed on the base by a process such as sputtering. Conductive materials for use in embodiments of the

25      invention include gold, silver, platinum, copper, chrome or the like. In certain embodiments of the invention, the base substrate is coated with a layer of chrome or titanium prior to the application of the conductive material in order to promote adhesion of the base substrate and the conductive layer. In some embodiments of the invention, the substrate is baked prior to disposing the sacrificial metal layer on to the positive

30      photoresist layer. Optionally in these methods, the negative photoresist layer and/or the

positive photoresist layer is applied to the substrate by spin coating.

One embodiment of the invention is a mold for forming a polymerized composition having a predetermined geometry comprising a metallic substrate capable of containing a polymerizable composition; where the polymerized composition produced

5 by the mold has an optimized aspect ratio geometry, the polymerized composition produced by the mold is between about 10 to 2000 microns in thickness; and the mold has sufficient mechanical strength to withstand release of a polymerized poly(dimethylsiloxane) composition without breaking. A related embodiment of the invention is a method of making a mold for forming a polymerized composition of a

10 predetermined geometry comprising: providing a base substrate; disposing a conductive layer on to (a portion) the base substrate; disposing a positive photoresist layer on to the conductive layer; disposing a sacrificial metal layer on to the positive photoresist layer; disposing a negative photoresist layer on to the sacrificial metal layer; developing the negative photoresist layer via UV photolithography; removing the areas of the sacrificial

15 metal layer exposed by the development of the negative resist layer using an etchant; exposing these components to UV photolithography (with or without the use of a photomask); developing the positive photoresist layer via a developing solvent; electroplating these components with a layer of conductive material; and then removing the positive photoresist layer, the sacrificial metal layer, and the negative photoresist layer

20 from the so layered substrate using a solvent so that the mold is made. Typically, the mold made by the method can be used repeatedly. Optionally in this method, the base substrate is coated with a layer of chrome or titanium prior to the application of the conductive layer in order to promote adhesion of the base substrate and the conductive layer. Optionally, the substrate is baked prior to disposing the sacrificial metal layer on

25 to the positive photoresist layer. Optionally the negative photoresist layer and/or the positive photoresist layer is applied to the substrate by spin coating.

### III. METHODS FOR USING ANALYTE SENSOR APPARATUS OF THE INVENTION

30

A related embodiment of the invention is a method of sensing an analyte within

the body of a mammal, the method comprising implanting an analyte sensor

embodiment disclosed herein in to the mammal and then sensing an alteration in current

at the working electrode and correlating the alteration in current with the presence of the

analyte, so that the analyte is sensed. Typically the analyte sensor is polarized anodically

5       such that the working electrode where the alteration in current is sensed is an anode. In

one such method, the analyte sensor apparatus senses glucose in the mammal. In an

alternative method, the analyte sensor apparatus senses lactate, potassium, calcium,

oxygen, pH, and/or any physiologically relevant analyte in the mammal.

        Certain analyte sensors having the structure discussed above have a number of

10      highly desirable characteristics which allow for a variety of methods for sensing analytes

in a mammal. For example in such methods, the analyte sensor apparatus implanted in

the mammal functions to sense an analyte within the body of a mammal for more than 1,

2, 3, 4, 5, or 6 months. Typically, the analyte sensor apparatus so implanted in the

mammal senses an alteration in current in response to an analyte within 15, 10, 5 or 2

15      minutes of the analyte contacting the sensor. In such methods, the sensors can be

implanted into a variety of locations within the body of the mammal, for example in both

vascular and non-vascular spaces.


IV.     KITS AND SENSOR SETS OF THE INVENTION

20      In another embodiment of the invention, a kit and/or sensor set, useful for the

sensing an analyte as is described above, is provided. The kit and/or sensor set typically

comprises a container, a label and an analyte sensor as described above. Suitable

containers include, for example, an easy to open package made from a material such as a

metal foil, bottles, vials, syringes, and test tubes. The containers may be formed from a

25      variety of materials such as metals (e.g. foils) paper products, glass or plastic. The label

on, or associated with, the container indicates that the sensor is used for assaying the

analyte of choice. In some embodiments, the container holds a porous matrix that is

coated with a layer of an enzyme such as glucose oxidase. The kit and/or sensor set may

further include other materials desirable from a commercial and user standpoint,

30      including elements or devices designed to facilitate the introduction of the sensor into

the analyte environment, other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

Various citations are referenced throughout the specification. In addition, certain text from related art is reproduced herein to more clearly delineate the various embodiments of the invention. The disclosures of all citations in the specification are expressly incorporated herein by reference.

| TABLE 1 | | | | |
|---|---|---|---|---|
| Lot 3422<br>Plates 1-2<br>2x 3x | Lot 3422<br>Plates 3-4<br>2x 3x | Lot 3422<br>Plates 5-6<br>2x3x | Lot 3422<br>Plates 7-8<br>2x 3x | Lot 3422<br>Plates 9-10<br>2x 3x |
| Standard metal plating for 2x3x electrodes. (100% current density) | Cyclic metal plating 120 sec, -36 uA, 160 sec, -54uA, 120 sec, -36uA just for working electrode. Counter and ref electrodes get std plating for 2x3x config. | Cyclic metal plating 120 sec, -36 uA, 160 sec, -54 uA, 60 sec, -36 uA just for working electrode. Counter and ref electrodes get std plating for 2x3x config. | Cyclic metal plating 60 sec, -36 uA, 160 sec, 54 uA, 120 sec, -36 uA just for working electrode. Counter and ref electrodes get std plating for 2x3x config. | Cyclic metal plating 60 sec, -36 uA, 160 sec, -54 uA, 60 sec, -36 uA just for working electrode. Counter and ref electrodes get std plating for 2x3x config. |
| Plasma 200W, 15s | Plasma 200W, 15s | Plasma 200W, 15s | Plasma 200W, 15s | Plasma 200W, 15s |
| spin Gox at 500rpm | spin Gox at 500rpm | spin Gox at 500rpm | spin Gox at 500rpm | spin Gox at 500rpm |
| x-link 2 hr, room temperature, rinse 30 min | x-link 2 hr., room temperature, rinse 30 min | x-link 2 hr., room temperature, rinse 30 min | x-link 2 hr., room temperature, rinse 30 min | x-link 2 hr., room temperature, rinse 30 min |
| spin AP10%-Ethanol no water, 2000rpm | spin AP10%-Ethanol no water, 2000rpm | spin AP10%-Ethanol no water, 2000rpm | spin AP10%-Ethanol no water, 2000rpm | spin AP10%-Ethanol no water, 2000rpm |
| Room temperature glut -2.5 hr rinse 30 min | Room temperature glut -2.5 hr rinse 30 min | Room temperature glut -2.5 hr rinse 30 min | Room temperature glut -2.5 hr rinse 30 min | Room temperature glut -2.5 hr rinse 30 min |
| Test 2 sensors per plate, then surface area test | Test 2 sensors per plate, then surface area test | Test 2 sensors per plate, then surface area test | Test 2 sensors per plate, then surface area test | Test 2 sensors per plate, then surface area test |
| Standard GLM 169kD at 0.9 yos, 31 rpts | Standard GLM 169kD at 0.9 yos, 31 rpts | Standard GLM 169kD at 0.9 yos, 31 rpts | Standard GLM 169kD at 0.9 yos, 31 rpts | Standard GLM 169kD at 0.9 yos, 31 rpts |
| NO HPM | NO HPM | NO HPM | NO HPM | NO HPM |
| Test 1 sensor per plate | Test 1 sensor per plate | Test 1 sensor per plate | Test 1 sensor per plate | Test 1 sensor per plate |
| Assemble as –003 and sterilize | Assemble as -003 and sterilize | Assemble as -003 and sterilize | Assemble as -003 and sterilize | Assemble as -003 and sterilize |

| TABLE 2 | | |
|---|---|---|
| Lot 3422 Plates 11-22 2x 3x-Control | Lot 3422 Plates 13-14 2x3x | Lot 3422 Plates 15-16 2x 3x |
| Standard metal plating for 2x3x electroces. (100% current density) | Cyclic metal plating 60 sec @ -36 uA, 120 sec @ -58 uA, 120 sec @ -36 uA for working electrode. Counter and ref electrodes get std plating for 2x3x config. | Cyclic metal plating 60 sec @ -36 uA, 120 sec @ -58 uA, 120 sec @ -36 uA, for working electrode. Plate Counter for 60 sec @ 72uA, 120 sec @ 96 uA and 120 sec @ 72uA. Ref electrodes get std plating. |
| Plasma 200W, 15s | Plasma 200W, 15s | Plasma 200W, 15s |
| spin Gox at 500rpm | spin Gox at 500rpm | spin Gox at 500rpm |
| x-link 2 hr, room temperature, rinse 30 min | x-link 2 hr, room temperature, rinse 30 min | x-link 2 hr, room temperature, rinse 30 min |
| spin adhesion promoter (AP) 10%- Ethanol no water, 2000rpm | spin AP10%- Ethanol no water, 2000rpm | spin AP10%- Ethanol no water, 2000rpm |
| Room temperature glutaraldehyde -2.5 hr rinse 30 min | Room temperature glut -2.5 hr rinse 30 min | Room temperature glut -2.5 hr rinse 30 min |
| Test 2 sensors per plate, then surface area test | Test 2 sensors per plate, surface area test | Test 2 sensors per plate, then surface area test |
| Standard GLM 169kD at 1.0 yos, 31 rpts | Standard GLM 169kD at 1.0 yos, 31 rpts | Standard GLM 169kD at 1.0 yos, 31 rpts |
| NO HPM | NO HPM | NO HPM |
| Test 1 sensor per plate | Test 1 sensor per plate | Test 1 sensor per plate |
| Assemble as -003 and sterilize | Assemble as -003 and sterilize | Assemble as -003 and sterilize |

CLAIMS:

1.      A method of performing an electrochemical reaction within an analyte sensor,
the method comprising:

5        using an analyte sensor constructed to perform an electrochemical reaction when
exposed to an analyte, wherein:

        the analyte sensor includes at least one electrode disposed upon a base substrate;
and

        the base substrate includes a geometric feature selected to increase the surface

10      area of an electrochemically reactive surface on the electrode disposed thereon such that
surface area to volume ratio of the electrochemically reactive surface area of the
electrode disposed on the geometric feature is greater than surface area-to-volume ratio
of the reactive surface of the electrode when disposed on a flat surface; and

        exposing the analyte sensor to an analyte so that a electrochemical reaction is

15      performed within the analyte sensor.

2.      The method of claim 1, wherein the geometric feature is a lip, a shoulder, a ridge,
a notch, a depression or a channel.

20      3.      The method of claim 1, wherein the analyte sensor comprises a plurality of
discrete geometric features having a plurality of electrochemically reactive electrode
surfaces.

4.      The method of claim 3, wherein the analyte sensor comprises at least 2, 3, 4, 5, 6,

25      7, 8, 9 or 10 discrete geometric features having a plurality of electrochemically reactive
electrode surfaces.

5.      The method of claim 1, wherein the electronic signal in response to exposure to
the analyte that is generated at the electrochemically reactive surface area of the electrode

30      disposed on the geometric feature is greater than an electronic signal generated an

electrochemically reactive surface area of the electrode when the electrode is disposed on a flat surface.

6.    The method of claim 1, wherein the analyte sensor is designed to be implantable within a mammal.

7.    The method of claim 1, wherein the in vivo lifetime of the analyte sensor having the electrochemically reactive surface area of the electrode disposed on the geometric feature is greater than the in vivo lifetime of an analyte sensor where an electronic signal is generated on an electrochemically reactive surface area of an electrode when the electrode is disposed on a flat surface.

8.    The method of claim 1, wherein the geometric feature causes the electrochemically reactive surface area of the electrode to form a nodule.

9.    The method of claim 1, wherein the implantable analyte sensor comprises
        an analyte sensing layer disposed on the electrode, wherein the analyte sensing layer
detectably alters the electrical current at the electrode in the presence of an analyte;
        an optional protein layer disposed on the analyte sensing layer;
        an adhesion promoting layer disposed on the analyte sensing layer or the optional protein layer, wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer; and
        an analyte modulating layer disposed on the analyte sensing layer, wherein the analyte modulating layer modulates the diffusion of the analyte therethrough; and
        an optional cover layer disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer.

10.     The method of claim 9, wherein the analyte sensing layer comprises a protein reactive with an analyte present in mammalian blood.

11.     The method of claim 10, wherein the protein is glucose oxidase, glucose

5     dehydrogenase, lactate oxidase, hexokinase or lactate dehydrogenase.

12.     The method of claim 1, wherein in the electrochemical reaction, hydrogen peroxide is oxidized at the electrochemically reactive surface area of the electrode disposed on the geometric feature.

10

13.     The method of claim 1, wherein the surface area to volume ratio of the electrochemically reactive surface area of the electrode disposed on the geometric feature is at least 10%, 25%, 50%, 75% or 100% greater than surface area-to-volume ratio of the reactive surface of the electrode when disposed on a flat surface.

15

14.     An analyte sensor for detecting an analyte in a fluid, the apparatus comprising:
        at least one electrode disposed upon a base substrate,
wherein the base substrate includes a geometric feature selected to increase the surface area of an electrochemically reactive surface on the electrode deposited thereon such that

20     surface area to volume ratio of the electrochemically reactive surface area of the electrode disposed on the geometric feature is greater than surface area-to-volume ratio of the reactive surface of the electrode when disposed on a flat surface.

15.     The apparatus of claim 14, wherein the geometric feature is a lip, a shoulder, a

25     ridge, a notch, a depression or a channel.

16.     The apparatus of claim 14, wherein the analyte sensor comprises a plurality of discrete geometric features having a plurality of electrochemically reactive electrode surfaces.

30

17.    The apparatus of claim 14, wherein the electronic signal in response to exposure to the analyte that is generated at the electrochemically reactive surface area of the electrode disposed on the geometric feature is greater than an electronic signal generated an electrochemically reactive surface area of the electrode when the electrode is disposed

5    on a flat surface.

18.    The apparatus of claim 14, wherein the analyte sensor is designed to be implantable within a mammal.

10    19.    The apparatus of claim 18, wherein the in vivo lifetime of the analyte sensor having the electrochemically reactive surface area of the electrode disposed on the geometric feature is greater than the in vivo lifetime of an analyte sensor having an electronic signal generated an electrochemically reactive surface area of an electrode when the electrode is disposed on a flat surface.

15

20.    The apparatus of claim 14, wherein the implantable analyte sensor comprises
        an analyte sensing layer disposed on the electrode, wherein the analyte sensing
layer
detectably alters the electrical current at the electrode in the presence of an analyte;
20            an optional protein layer disposed on the analyte sensing layer;
        an adhesion promoting layer disposed on the analyte sensing layer or the optional
protein layer, wherein the adhesion promoting layer promotes the adhesion between the
analyte sensing layer and an analyte modulating layer disposed on the analyte sensing
layer; and
25            an analyte modulating layer disposed on the analyte sensing layer, wherein the
analyte modulating layer modulates the diffusion of the analyte therethrough; and
        an optional cover layer disposed on at least a portion of the analyte modulating
layer, wherein the cover layer further includes an aperture over at least a portion of the
analyte modulating layer.

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21.     A method of modulating electrochemical reactions within an implantable analyte
sensor, the method comprising performing electrochemical reactions within an
implantable analyte sensor comprising:
        a working electrode having a reactive surface area, wherein during analyte
5       sensing, the working electrode generates electrons that reduce a plurality of composition
species in the electrochemical reaction including oxygen ($O_2$); and
        a counter electrode having a reactive surface area, wherein the size of the reactive
surface area of the counter electrode is selected so as to control the reduction of the
plurality of composition species in the electrochemical reaction so that oxygen ($O_2$) is the
10      predominant composition species reduced by the electrons generated at the working
electrode;
        so that electrochemical reactions within the implantable analyte sensor are
modulated.

15      22.     The method of claim 21, wherein the surface area of the counter electrode is 1.5,
2, 2.5 or 3 times the size of the working electrode.

23.     The method of claim 21, wherein the implantable analyte sensor comprises
        an analyte sensing layer disposed on the working electrode, wherein the analyte
20      sensing layer
detectably alters the electrical current at the working electrode in the presence of an
analyte;
        an optional protein layer disposed on the analyte sensing layer;
        an adhesion promoting layer disposed on the analyte sensing layer or the optional
25      protein layer, wherein the adhesion promoting layer promotes the adhesion between the
analyte sensing layer and an analyte modulating layer disposed on the analyte sensing
layer; and
        an analyte modulating layer disposed on the analyte sensing layer, wherein the
analyte modulating layer modulates the diffusion of the analyte therethrough; and
30      an optional cover layer disposed on at least a portion of the analyte modulating

layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer.

24.    The method of claim 21, wherein the working electrode and the counter electrode comprise a micro-porous matrix.

25.    The method of claim 24, wherein the micro-porous matrix has a surface area that is at least 2, 4, 6, 8, 10, 12, 14, 16 or 18 times the surface area of a non-porous matrix of same dimensions.

26.    The method of claim 22, wherein the analyte sensing layer is a protein.

27.    The method of claim 26, wherein the protein is glucose oxidase, glucose dehydrogenase, lactate oxidase, hexokinase or lactate dehydrogenase.

28.    The method of claim 21, wherein hydrogen peroxide is oxidized at the working electrode.

29.    The method of claim 22, wherein the implantable analyte sensor further comprises an interference rejection layer disposed between the surface of the working electrode and the analyte sensing layer.

30.    An implantable electrochemical analyte sensor comprising:

a working electrode having a reactive surface area, wherein during analyte sensing, the working electrode generates electrons that reduce a plurality of composition species in the electrochemical reaction including oxygen ($O_2$); and

a counter electrode having a reactive surface area, wherein the size of the reactive surface area of the counter electrode is selected so as to control the reduction of the plurality of composition species in the electrochemical reaction so that oxygen ($O_2$) is the predominant composition species reduced by the electrons generated at the working

electrode;

an analyte sensing layer disposed on the working electrode, wherein the analyte sensing layer

detectably alters the electrical current at the working electrode in the presence of an

5     analyte;

an adhesion promoting layer disposed on the analyte sensing layer or the protein layer, wherein the adhesion promoting layer promotes the adhesion between the analyte sensing layer and an analyte modulating layer disposed on the analyte sensing layer; and

an analyte modulating layer disposed on the analyte sensing layer, wherein the

10    analyte modulating layer modulates the diffusion of the analyte therethrough; and

a cover layer disposed on at least a portion of the analyte modulating layer, wherein the cover layer further includes an aperture over at least a portion of the analyte modulating layer.

15    31.    A method of making a metallic electrode using cycles of differing electroplating conditions, the method comprising:

(a) electroplating a metal composition onto a substrate under a first set of conditions selected to produce a first metal layer having a first surface area and a first adhesion strength between the substrate and the first metal layer; and

20    (b) electroplating a metal composition onto the first metal layer under a second set of conditions selected to produce a second metal layer having a second surface area and a second adhesion strength between the first metal layer and the second metal layer, wherein:

(i) the second set of conditions produces a second metal layer having a second

25    surface area that is greater than the first surface area of the first metal layer produced by the first set of conditions; and

(ii) the second set of conditions produces a second metal layer having an adhesion with the first metal layer that is greater than the adhesion between the first metal layer and the substrate produced by the first set of conditions;

so that the metallic electrode is made using cycles of differing electroplating conditions.

32.     The method of claim 31, further comprising: (c) electroplating a metal composition onto the second layer under a third set of conditions selected to produce a third metal layer having a third surface area.

33.     The method of claim 32, wherein the third set of conditions produces third metal layer having a greater density than the density of the second metal layer.

34.     The method of claim 32, wherein the third set of conditions produces a third metal layer having a third surface area that is less than the second surface area of the second metal layer produced by the second set of conditions.

35.     The method of claim 31, wherein the substrate does not comprise platinum.

36.     The method of claim 31, wherein the substrate comprises gold.

37.     The method of claim 31, wherein the electroplated metal composition comprises platinum black.

38.     The method of claim 31, wherein the substrate comprises a geometric feature selected to increase the surface area of the electroplated metal composition.

39.     The method of claim 31, wherein a metal composition is electroplated on to a porous substrate.

40.     The method of claim 31, wherein the substrate comprises a planar surface and an edge or lip at the boundary of the planar surface and the cycles of differing electroplating conditions further inhibit an uneven deposition of the metal layer electrodeposited onto the planar surface and the edge or lip at the boundary of the planar surface.

41.     The method of claim 40, wherein the uneven deposition of the metal layer that is inhibited is a greater deposition of metal on the edge or lip at the boundary of the planar surface relative to the deposition of metal on the planar surface.

42.     The method of claim 31, wherein the first metal layer electroplated under a first set of conditions exhibits a resistance to abrasion from the substrate that is greater than

the resistance to abrasion exhibited by a metal layer electroplated to the substrate under the second set of conditions.

43.     The method of claim 32, wherein surface area of the third layer is at least 160, 170 or 180 times the geometric area of the third layer.

5     44.     The method of claim 32, wherein surface area of the third layer is 230 – 260 times the geometric area of the third layer.

45.     An implantable biosensor comprising:

(a) an electrode comprising a plurality of electrodeposited metal layers including:

(ii) a first metal layer having a first surface area and a first adhesion

10     strength with a substrate on which the first layer is electrodeposited; and

(ii) a second metal layer deposited on the first metal layer, the second metal layer having a second surface area and a second adhesion strength with the first layer on which the second layer is electrodeposited, wherein the second surface area is greater than the first surface area and the second adhesion strength

15     is greater than the first adhesion strength; and

(b) an enzyme layer disposed on the electrode, wherein an enzyme in the enzyme layer is capable of reacting with and/or producing a molecule whose change in concentration can be measured by measuring a change in the current at the electrode.

46.     The implantable biosensor of claim 45, wherein the electrode comprises a third

20     metal layer deposited on the second metal layer, wherein the third metal layer has a greater density than the density of the second metal layer.

47.     The implantable biosensor of claim 45, wherein the enzyme layer comprises glucose oxidase or lactate oxidase.

48.     The implantable biosensor of claim 41, wherein the biosensor is a glucose

25     biosensor.

49.     An electrode comprising a plurality of electrodeposited metal layers comprising:

(a) a first metal layer having a first surface area;

(b) a second metal layer deposited on the first metal layer, the second metal layer having a second surface area that is greater than the first surface area of the first metal layer; and

(c) a third metal layer deposited on the second metal layer, the third metal layer having a greater density than the density of the second metal layer.

50.     The electrode of claim 48, wherein the substrate comprises a geometric feature selected to increase the surface area of the electrodeposited metal composition.
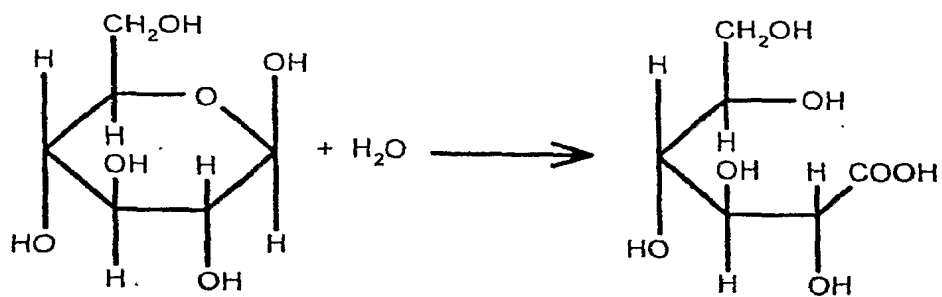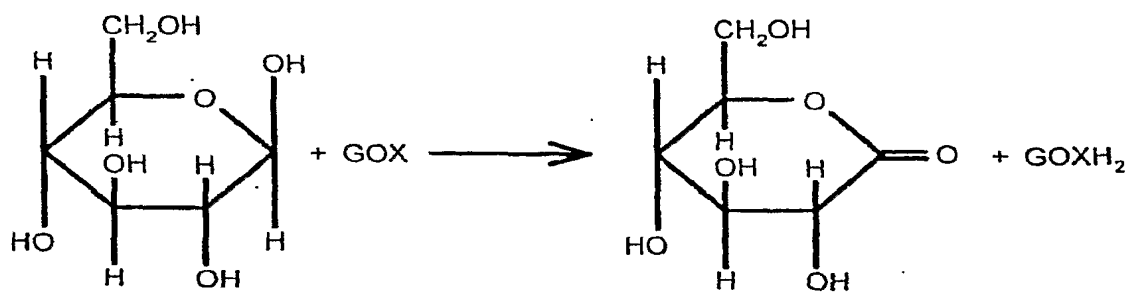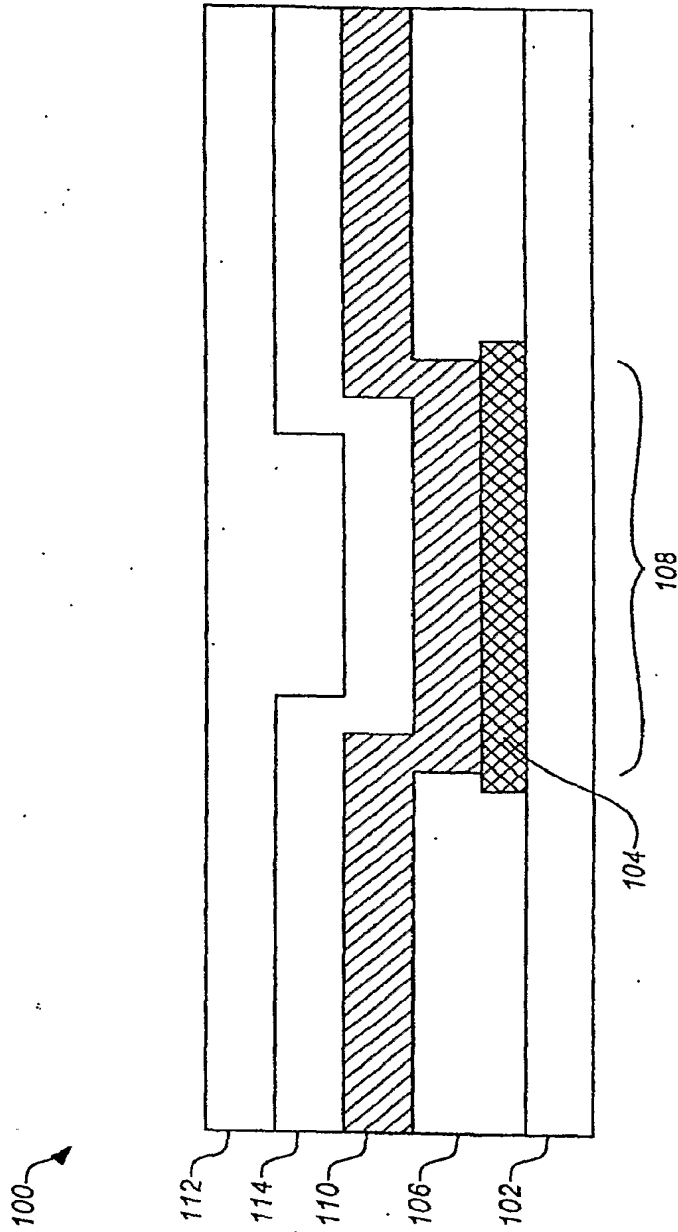
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FIG. 1

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FIG. 2